James T. Barrett

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LECTURES ON PLANT PHYSIOLOGY

BY

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AUTHORIZED ENGLISH TRANSLATION

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PREFACE TO THE GERMAN EDITION

Of late years the subject of Plant Physiology has been discussed in a succession of admirable treatises. In addition to Pfeffer's Handbook, which laid the foundation of *modern* Plant Physiology, there are the briefer expositions of the subject in the textbooks of General Botany, such as those of Noll and Wiesner, but a textbook of Plant Physiology of moderate size has been for long a desideratum. The object of the present volume is to introduce the subject to those who are already familiar with the fundamentals of natural science, and inasmuch as it takes the form of lectures which I have been in the habit of giving in this University for a number of years, and is the direct outcome of them, I have styled the book *Lectures* on Plant Physiology.

The characteristic feature of such a textbook, apart from the mere mode of presentation, must lie in the selection and arrangement of its subject-matter, and those who are familiar with the works of others will be able to see at once wherein the present treatise differs from them. It is unnecessary for me to refer here to the general line of argument of the book, since that may be readily grasped from a study of the table of contents. Still less need I attempt to justify it, for if it does not speak for itself, it will not help matters to go into a detailed explanation of the principles of arrangement and selection in a preface. I may, therefore, confine myself to saying a few words on the treatment of the literature and on the illustrations.

A bibliography will be found at the end of each lecture. This contains, in addition to works of fundamental importance on the subject more immediately concerned, references to numerous special papers recognized as the authoritative statements for individual observations or views. That the selection of the literature must be arbitrary goes without saying ; for it would be possible in the long run to cite one or more authorities for almost every word I have written. The place which Pfeffer's Handbook has taken in modern botanical literature has rendered it necessary to quote from it on almost every page ; rather than pursue this course, the present general reference must suffice; where, however, it has been specially referred to, the citation is made in the abbreviated form, ' Pfeffer, Phys.' (Pfeffer, W. Pflanzenphysiologie, ein Handbuch der Lehre vom Stoffwechsel und Kraftwechsel in der Pflanze. Bd. I, 2. Aufl., Leipzig, 1897 ; Bd. II, 1. Hälfte, 2. Aufl., Leipzig, 1901 ; Bd. II, 1. Aufl., Leipzig, 1881.) Other works are indicated in the text, as a rule, by the author's name and the year of publication only. The dates are intended to serve merely as

reference numbers to the authorities cited at the end of the lecture dealing with the subject, and must not be taken as in any sense indicating the date of the discovery of a fact or of the establishment of a theory. I have placed the more recent literature prominently in the foreground, simply because, with its aid, the student can have no difficulty in tracing the older works on the subject; I do not desire, by this method of citation, to imply that modern works only are worthy of consideration. It has not been possible to enter into the history of the science, save only in a few places where it seemed essential to the elucidation of the subject.

Only a few of the illustrations are original; the great majority are reproductions from memoirs or textbooks, and for their execution I am greatly indebted to Fräulein A. Winnecke. A number of clichés have been borrowed from the works of Detmer, Fischer, Goebel, Klebs, Küster, Strasburger, and Verworn, as well as from the 'Bonn Textbook' (Lehrb. d. Botanik. Strasburger, Noll, Schenck, u. Schimper). To the authors of these works my best thanks are due for permission to use these illustrations.

LUDWIG JOST.

STRASSBURG, i. E., *November*, 1903.

AUTHOR'S PREFACE TO THE ENGLISH EDITION

It is both a pleasure and a duty to express to my friend, Professor Harvey Gibson, of the University of Liverpool, my sincere thanks for having made my Lectures on Plant Physiology more readily accessible to English readers through the medium of the present translation.

I have taken the opportunity of making brief alterations, here and there, in the original German text, with the view of correcting errors or removing ambiguities. In order to bring the work up to date, and to indicate the more important additions to the science that have been made since the original manuscript was completed (spring of 1903), supplementary notes, distinguished by being enclosed in square brackets, have been appended to or introduced into each lecture. Obviously it is not possible to do more than offer a selection of the extensive current literature in the present translation any more than in the original text. Czapek's Biochemie (Jena, 1905, vol. ii) is a work of special importance in relation to Part I, and reference must be constantly made to it. Similarly with regard to Part II, Pfeffer's Plant Physiology, vol. ii (Leipzig, 1904), will be found to be equally invaluable as a work of reference. Since the English translation of that treatise has been already published, it appeared to me unnecessary to introduce special references to it. All those who desire to study the subject of plant physiology more closely are strongly recommended to consult that work.

LUDWIG JOST.

Strassburg, 1906.

TRANSLATOR'S PREFACE TO THE ENGLISH EDITION

I HAVE been prompted to undertake a translation of Professor Jost's Vorlesungen über Pflanzenphysiologie by the desire to see made available for English-speaking students a critical modern exposition of so important and progressive a subject, moderate in size, adequately full in detail, and written in a lucid and interesting manner by a teacher of Dr. Jost's rank and experience.

I have not attempted to 'edit' Dr. Jost's pages in any way, feeling that the author should be allowed to speak for himself without comments and interpolations on the part of his translator. Such additions to and alterations in the German edition as have been made are from Dr. Jost's own pen, and are indicated, as he has said, by enclosure within square brackets. At the Author's request, I have endeavoured to translate the German text as literally as was possible without, I hope, unduly offending against the rules and idioms of the English language. Under the circumstances, of course, it will be readily understood that absence of critical comment does not signify concurrence with every view put forward by the Author, or complete acquiescence in his mode of treatment of every subject.

I have to acknowledge my indebtedness and to offer my grateful thanks to several friends who have aided me in matters of interpretation or expression, but more especially to Professor I. Bayley Balfour, F.R.S., who has been so kind as to look through the proof-sheets, and to furnish me with many valuable suggestions and criticisms. I am also indebted to my colleague, Dr. A. W. Titherley, for much help in the interpretation of the German names for organic and inorganic chemical compounds.

R. J. H. G.

LIVERPOOL, 1907.

SUMMARY OF CONTENTS

Introduction

Problems of Physiology, 1 ; Methods of Physiology, 2.

PART I

METABOLISM

I. Material Composition of the Plant

Chemical analysis, 3. Microscopical analysis, 6. Structure of the cell, 7 ; chemistry of the cell, more especially of the protoplasm, 8.

II. Absorption of Materials in General

Osmosis, 11. Osmotic characters of protoplasm, 12. Impermeability, 12 ; osmotic pressure, 13 ; permeability, 13 ; storage, 20 ; selective power, 20.

III. Absorption of Materials in detail. Fate of the Substances absorbed

CHAPTER I

Water

1. Significance of water, 24.
2. Absorption of water. Through the root ; characters of the soil, 25 ; characters of the root, 26 ; external influences, 31. Through aerial organs, 32.
3. Excretion of water. Transpiration. Proof, 35 ; cuticular and stomatal transpiration, 38 ; external influences, 38 ; stomata, 39 ; retardation of transpiration, 42 ; promotion of transpiration, 42 ; significance of transpiration, 43. Excretion of liquid water, 50 (see under 4).
4. Conduction of water. Organs for the conduction of water. Cells, 45 ; vessels, 47 ; filling of vessels with water, 49. Bleeding, 50 ; conditions, 53 ; causes, 54. Excretion of water by hydathodes, 58 ; significance of the excretion of liquid water, 59. Mode of conduction of water. Problems connected with the quantity of water conducted, 61 ; root pressure as a cause of the conduction of water, 63 ; leaf suction as a cause of the conduction of water, 64 ; physical questions, 64 ; peculiarities of the plant, 66 ; Jamin's chain, 71 ; influence of living cells, 74.

CHAPTER II

Mineral Constituents

1. Source of the mineral constituents, 77.
2. Absorption of the mineral constituents, 78.
3. Significance of the mineral constituents, 80; necessity for certain elements, 80; evidence for this necessity, especially by means of water culture, 81 ; significance of the essential elements, 83 ; unessential nature of other elements, 86 ; stimulants, 87.

4. The mineral constituents of the soil. The soil. Origin, 90; mineral contents, 91; absorption, 92. The action of the plant on the soil. Union of root-hairs and soil particles, 94; excretion of acids, 95; renewal of root-hairs, 96; branching of the root, 97.

5. Soil and plant distribution. Physico-chemical causes of plant distribution, 97; association, 99; historical causes, 100.

6. Soil and Agriculture, 101.

CHAPTER III

Carbon and Nitrogen

1. Assimilation in Holophytic Plants and Fate of the Products of Assimilation

a. Assimilation of Carbon.

1. The fundamental facts. Proof of the decomposition of carbon-dioxide. Air-bubble method, 104; gas-analysis method, 105; other methods, 105. Significance of chlorophyll, 106; physical and chemical characters of chlorophyll, 107. Concomitant action of sunlight, 110. Products of assimilation. Nature, 110; amount, 114.

2. Influence of external factors on carbon-dioxide assimilation. Influence of carbon-dioxide. Carbon-dioxide in air, soil, water, 118; influence of concentration, 119; entry into the plant, 120. Influence of other factors; (1) indirect, through modification of the plant, 121; (2) direct influence of oxygen, 124; of temperature, 124; of light, 125; intensity of light, 125; wave-length, 126; light as a source of energy, 129; light becomes absorbed, 129.

3. Historical data as to the assimilation of carbon-dioxide, 131.

b. Assimilation of Nitrogen. Sources of nitrogen, 133; gain and loss of nitrogen in nature, 135. Absorption of nitrogen, 137. Assimilation of nitrogen. Construction of proteid, 138; chemistry of proteid, 138; assimilation of nitric acid, 141; of ammonia, 142; organic compounds of nitrogen, 143. Assimilation of sulphur and phosphorus, 145.

c. Fate of the products of assimilation.

1. Solution of reserves:—(*a*) in seeds, 148; starch, 149; diastase, 150; enzymes, 152; catalytic phenomena, 152; action of enzymes on hydrogen peroxide, 152; incompleteness of the enzyme reaction, 153; dissolution of starch in seeds, 154; removal of the embryo, 156; inhibitory agents, 156; cellulose, 157; cell-wall, 157; cytase, 158; fats, 158; chemistry, 158; decomposition, 159; proteid, 159; pepsin and trypsin, 160; (*b*) in perennials, 162; (*c*) in trees, 163; (*d*) in foliage leaves, 164.

2. Circulation of the dissolved reserves. Migration from the leaf, 166; from other storehouses of reserves, 167. Causes of the translocation. Diffusion, 167; accelerating agents, 167. Organs concerned in the transport of material. Parenchyma, 170; sieve-tubes, 170; vessels, 172. Objects of translocation, 172.

3. Fate of the translocated materials. Transformation into plasta, 173. Formation of reserves. Carbohydrates, 173; proteid, 173; fat, 175. Formation of waste products, 176.

2. Acquisition of Carbon and Nitrogen by Heterotrophic Plants

a. Saprophytes.

1. Acquisition of carbon, 177; nutritive value of different sources of carbon, 178; omnivors and specialists, 180.

2. Acquisition of nitrogen, 181.

3. Saprophytes in nature, 182; dead organisms, humus, 183; excretion of enzymes, 183.

b. Insectivorous plants, 184.

c. Parasites, 186.

d. Metabolism in heterotrophic plants, 188.

3. Katabolism in Autotrophic and Heterotrophic Plants

a. Respiration. Decrease in dry weight, 191; economic coefficient, 191. Extent of respiration. Proof, 191; respiration in chlorophyll-containing cells, 194. Respiratory materials. Carbohydrates: complete combustion, 196; formation of acids, especially in Fungi and succulents, 197; fats, 199; proteid, 200. Dependence on external factors. Light and temperature, 201; influence of materials, 201. Intra-molecular respiration, 202; cause (204) and significance (205) of respiration. Historical data, 205.

b. Fermentation, 207.

1. Alcoholic fermentation, especially of yeast, 207; nutritive materials, 208: fermentative materials of yeast, 208; products of fermentation, 210; cause of fermentation, 211; zymase, 211; relation to enzymes, 211; zymase in intra-molecular respiration, 212. Dependence on oxygen, 212; aerobes, anaerobes, 213. Biological significance of fermentation, 213.
2. Butyric acid fermentation, 214; effect of oxygen, 215.
3. Further fermentation of alcohol into acetic and carbonic acids, 216.
4. Fermentation of carbohydrates, 217; formation of lactic acid, 217; formation of butyric acid, 217; fermentation of cellulose, 217; fermentation of pectin, 218. Conversion of the products of fermentation by other organisms, 218.
5. Fermentation of proteids, 219.

4. Special Cases of Anabolism and Katabolism

a. Oxidation of inorganic materials (respiration).

1. Sulphur-bacteria. *Beggiatoa*. Origin of sulphuretted hydrogen, 221; oxidation of sulphuretted hydrogen, 221; red sulphur bacteria, 223.
2. Iron-bacteria, 224.
3. Nitro-bacteria. Origin of ammonia. Fermentation of urea, 224; oxidation of ammonia, 225; nitrate and nitrite bacteria, 226.

b. Assimilation in nitro- and sulphur-bacteria. Assimilation of carbonic acid by nitro-bacteria, 227; oxidation of organic substances by nitro-bacteria, 228; assimilation in sulphur-bacteria, 229; sensitivity of nitro-bacteria to organic substances, 229.

c. Denitrification and fixation of nitrogen, 231.

1. Denitrification, 231.
2. Fixation of nitrogen, 232. *Clostridium pasteurianum*, 233; symbiotic union with other bacteria, 233; cultures, 234; other micro-organisms alleged to fix nitrogen, 234; Leguminosae, 235; symbiosis with *B. radicicola*, 237; fixation of nitrogen, 237.

d. Symbiosis and metabiosis.

1. Instances related to the case of Leguminosae, 239.
2. Mycorhiza. Endotrophic forms, 240; ectotrophic forms, 241.
3. Lichens, 242.
4. Metabiosis. Circulation of carbon and nitrogen in organisms, 243.

PART II

METAMORPHOSIS

I. Problems of Developmental Physiology

1. First example: development of the Myxomycetes, 247.
2. Second example: development of *Basidiobolus*, 248.
3. Third example: flowering plants. Development, 250; differentiation and division of labour, 250; correlations, 252. Influence of external conditions on development, 252; formal conditions, 253; formative results, 254.
4. Fundamental problems of Biology. Mechanical explanation of life, 254; organization and mechanism, 254; matter and form, 256.

II. Growth and Formation under Constant External Conditions

a. Growth of the cell, 258.

1. Growth, 258 ; formation, 259.
2. Growth of the protoplasm, 259.
3. Growth and formation of the cell-wall, 260. Origin of the cell-wall, 260. Surface growth, 260; mechanics, 261 ; apposition, 262 ; intussusception, 262 ; significance of turgor tension, 263. Growth in thickness, 265 ; cessation of growth, 267 ; resumé, 267.
4. Cell-division, 267 ; nuclear division (mitosis), 268 ; cell-wall formation, 269 ; arrangement of walls, 269 ; amitosis, 271; extent of division, 271.

b. Growth of the entire plant, 272.

1. Somatophytes, asomatophytes, 273 ; embryonic growth, 273. Position and activity of the growing point, 273; shoot (symmetry, 275; form, 276); leaf, 277 ; root, 279. Structure of the growing point, 279 ; simple cases, 279 ; arrangement of cells at the shoot apex, 280; at the point of origin of the leaf, 281 ; at the point of origin of the root, 283. Adventitious growing points, 284.
2. Growth in extent, 285. Longitudinal growth:—measurement of increase, 286; distribution of growth (root, 287 ; shoot, 290; leaf, 292); rate, 293 ; duration, 294. Growth in thickness, 294.
3. Internal development. Evolution of cell-form, 296 (tissue tension, 297); cell-wall, 297 ; cell-contents, 297.

III. Influence of External Conditions on Growth and Formation

A. The non-living environment.

General observations, 298.

1. Temperature, 299. Alteration in the rate of growth. Cardinal points of temperature, 299 ; supra-maximum, 300 ; infra-minimum, 300. Alteration in form, 301.
2. Light. Cardinal points, 302.
   a. Influence of the intensity of light. Influence on the rate of growth, 303. Formative influences. Etiolation, 304 ; causes, 306 ; significance, 306 ; other results, 307 ; form and structure, 307 ; correlative influences, 307 ; colour, 308 ; juvenile forms, 308.
   b. Influence of the direction of light on polarity and symmetry, 310.
   c. Influence of the quality of light, 311.
3. Gravity, 312 ; mass acceleration, 313 ; intensity, 313 ; direction, 313 ; morphogenic results, 313 ; influence on growth, 314.
4. Mechanical influences, 314.
5. Influences of materials, 315 ; deficiency of nutrients, 315 ; oxygen, 316 ; poisons, 316 ; chemical stimuli, 317 ; water, 317.

B. Other Organisms.

Association, 320 ; parasites, 321; fungus galls, 321; insect galls, 321; symbiosis, 324.

C. Parts of the same body

Correlations, 326.

a. Evidence from observation, 327.

b. Evidence from removal of parts, 327. Immediate results of wounding. Healing of wounds, 327 ; regeneration, 328 ; different types of reparation, 329 ; polarity in regeneration, 330 ; qualitative results, 330.

c. Functional adaptation, 330.

d. Transplantation, 332 ; in normal orientation, 332 ; in inverted orientation, 332; on other species, 333; quantitative results, 334; qualitative results, 334.

e. Causes of correlations. Influences of materials, 335 ; in growth in thickness, 335 ; in regeneration, 336. Mechanical influences. Mechanical theory of leaf arrangement, 337.

Conclusion : the facts determining plant form, 338.

IV. The Development of the Plant under the Influence of Internal and External Factors

CHAPTER I

Periodicity in Vegetative Life

a. Activity and rest, 341.

b. Daily periodicity, 342 ; after-effect, 343.

c. Annual periodicity, 343.

1. In longitudinal growth. Trees. Leaf-formation, 344 ; causes of the periodicity, 345; root-formation, 346. Perennials, 346. Tropical plants, 347. Qualitative changes. Scale-leaves, 348 ; bracts, 349.

2. In growth in thickness, 350.

d. Periodicity in development as a whole, 351.

CHAPTER II

Periodicity in Reproduction

Definition of reproduction, 353 ; types of reproduction, 355.

a. Causes of reproduction in lower plants, 355 ; examples, 356 ; general results of Klebs's researches, 357.

b. Reproduction in the higher plants, 358. Reproduction in ferns. Alternation of generations, 358 ; apogamy and apospory, 359 ; accessory reproductive organs, 359. Reproduction in flowering plants. Alternation of generations, 361 ; accessory reproductive organs, 362. Causes of reproduction. Continuous vegetative growth, 362 ; causes of flower formation, 363 ; causes of the formation of accessory reproductive organs, 365.

c. Significance of reproduction, 365 ; general significance, 365 ; significance of fertilization, 366. Cessation of the check to development as a consequence of fertilization, 367 ; deficiency of nuclein in the ovum, 367 ; deficiency of chromosomes, 368 ; deficiency in kinoplasm, 369 ; parthenogenesis, 369 ; stimuli to development, 370 ; merogeny, 371. Fertilization as amphimixis, 371. Hybrids, 372 ; (α) production, 372 ; appearance, 373 ; characters, 373 ; (β) second generation : segregation, 375 ; (γ) its significance in estimating the meaning of fertilization, 375.

CHAPTER III

Heredity and Variation

a. Heredity. Initials, 376.

1. Heredity in unicellular types, 376.

2. Heredity in multicellular types, 376 ; idioplasm, 377 ; idioplasm in the cell, 377 ; idioplasm in the entire plant, 379 ; somatic and germ-cells, 379 ; disappearance and origin of initials, 380.

b. Origin of species. Theory of descent, 383 ; Darwinian theory, 384.

1. Selection. Mode of action in formation of species, 384 ; definition of a species, 384 ; specific characters, 385 ; characters associated with adaptation and organization, 386. Mode of action in adaptation, 386.

2. Variation. Fluctuating variations, 387. Adaptation, 389 ; capacity for adaptation, 389 ; inheritance of acquired characters, 390. Mutation, 393.

PART III

TRANSFORMATION OF ENERGY

Conservation of energy in the organism, 397.

The forms of energy in the plant:

1. Heat, 398; production of heat, 399; dependence on the state of development and on external factors, 399; relation to respiration, 400; sources, 400; significance, 401.
2. Light, 401.
3. Electricity, 402.
4. Mechanical energy, 403; sources, 403. Passive movements, 405; active movements and their classification, 405.

I. Hygroscopic Movements

Types of the movement, 406.

a. Movements due to swelling.

1. Swelling, 406.
2. Movements due to swelling and contraction, 409; curvatures due to differential swelling of parts, 410; due to arrangement of cells, 410; due to stratification of the cell-wall, 411; due to striation in the cell-wall, 411. Twinings, 412. Torsions, 414. Biological significance of these movements, 414.

b. Movements due to the cohesion of imbibition water. Fern sporangia, 415; anthers, 416.

II. Variation and Nutation Movements

Osmotic pressure: amount, 418; action on the cell-wall, 419; movements due to osmotic pressure, 421.

Growth as a cause of movements, 421.

Performance of work during such movements, 421.

CHAPTER I

Ejaculatory Movements

Examples of ejaculatory movements, 422; tensions in single cells, 422; in tissues, 424.

Significance of external shocks, 426; autonomous and paratonic movements, 426; *Catasetum*, 426; general remarks on movements due to external stimuli, 427; classification, 428.

CHAPTER II

Paratonic Movements

A. Tropisms (Directive Movements)

a. Geotropism, 429.

1. In orthotropic organs. Proof, 430; occurrence, 431. Geotropic curvature. In the root, 431; in the stem, 433 (amputated stems, 435); after the cessation of longitudinal growth, 435. Mechanics of curvature, 435. Significance of gravity. Action of the stimulus, 436; duration of the stimulus, 437; intensity (439) and direction (439) of gravity. Preliminary effects of the stimulus. Perception and reaction, 441; hypotheses as to perception (Noll, 442; Němec, and Haberlandt, 443). Protoplasmic movement, 444; precedent chemical phenomena, 444.

2. In plagiotropic organs. Radial organs, 446; rhizomes and lateral roots, 446; disposition due to internal influences, 447; autotropism, 448; branches of trees, 449; disposition due to external influences, 450. Dorsiventral organs, 452; branches, 452; flowers, 452; leaves with nutation and variation movements, 453. Twining plants, 455; revolving movements, 456; twining, 458.

b. Heliotropism. Likeness to geotropism, 460.

1. In orthotropic organs. Positive heliotropism, 460; disposition to negative heliotropism, 462; significance of light intensity, 462; adaptibility to light, 463. Behaviour in nature, 464.
2. In plagiotropic organs. Leaves. Growth movements, 464; variation movements, 466; fixed light position, 466. Other organs, 467.
3. Precedent phenomena in stimulation. Separation of zones of perception and reaction. Grasses, 468; Malvaceae, 470; roots, 470. Precedent phenomena in perception, 471; direction or intensity of light, 471; liminal intensity, 473; latent period, 473; quality of light, 474; immediate cause of phototropic activity, 474. Other phenomena in the chain of stimulation, 474.

c. Combined action of geotropism and heliotropism, 476.

d. Other tropisms, 478.

1. Thermotropism, 478.
2. Electrotropism and galvanotropism, 480.
3. Chemotropism, 481; aerotropism, 484; hydrotropism, 485; orientation with reference to the substratum, 486.
4. Traumatotropism, 486.
5. Rheotropism, 486.

B. Nastic Curvatures (Bending Movements)

a. Haptotropism (transition from tropisms to nastic curvatures).

1. Tendrils, 487. Result of temporary contact. Tendrils which are sensitive on one or on all sides, 490; more exact estimate of contact stimulus, 490; other stimuli, 492; curvature, 492. Encircling of the support; production of continuous twining, 494; further results, 494.
2. Leaf tendrils, climbing roots, *Cuscuta*, 495.
3. *Drosera*, 496. Character of the movement induced, 496. Stimuli and reactions, 497; direct stimulation, 497; indirect stimuli, 498.
4. Heat stimuli in haptotropic organs, 499.

b. Nyctitropism.

1. Paratonic movements, 501. Growth movements; flowers which react to changes in temperature (501) and in light (502); foliage leaves, 503. Variation movements, 503; mechanics, 505; influence of gravity, 506.
2. Periodic movements, 508; movements due to after-effects, 508; genesis of periodicity, 509; mechanics of periodic movements, 510.

c. Shock movements.

1. *Mimosa*, 512; character and significance of the movement, 513; preliminary changes in the articulation, 514; recurrence of the capacity for reaction, 515; estimation of the shock stimulus, 516; other stimuli, 516; transmission of the stimulus, 517.
2. Stamens of Cynareae, 519.
3. Other examples, 520.

C. Review of Paratonic Movements

Influence of external conditions on movements. Stimuli, 522. Releasing stimuli in mechanisms and organisms, 522; perception and reaction, 522; formal conditions, 526; general, 526; nature of their action, 527.

CHAPTER III

Autonomous Movements

Induced by internal stimuli, 528.

1. Autonomous variation movements, 528.
2. Autonomous growth movements, 529; circumnutation, 530; transitory and periodic nutation, 530; hyponasty and epinasty, 530; torsions and twinings, 531.

III. Locomotory Movements

CHAPTER I

Autonomous Locomotory Movements

Occurrence and classification, 532.

1. Natatory movements, 532.
2. Creeping movements, 534. Types of these: amoeboid movements, 534; rotation and circulation, 536. Causes of the movement, 536.
3. Formal conditions, 539.

CHAPTER II

Locomotory Directive Movements (Taxis)

a. Taxis in free organisms, 541.

1. Chemotaxis, 542; significance, 542; antherozoids of ferns, 542; other organisms, 544; strophic and apobatic reactions, 545; phenomena precedent to perception, 547.
2. Osmotaxis, 547; hydrotaxis, 548; rheotaxis, 548.
3. Thermotaxis, 548.
4. Phototaxis, 548; positive and negative reactions, 549; light direction or light intensity, 549; apobatic phototaxis, 550.
5. Galvanotaxis, 551.

b. Taxis in protoplasm and its organs, 552.

1. Phototaxis of chloroplasts, 552.
2. Traumatotaxis of the nucleus of the cell, 553.

INDEX 555

PART I. METABOLISM

LECTURE I

INTRODUCTION

Problems of Plant Physiology—Methods—Chemical composition and structure of the plant.

THE saying of the Greek philosopher—*πάντα ῥεῖ*—'everything is in a state of flux,' is in the highest degree applicable to the living organism, for continuous changes, both physical and chemical, are the inseparable characteristics of life, not only in the organism as a whole, but in the individual parts of which it is composed. Among these changes may be noted first those occurring in the non-living body; for instance, expansion as a result of exposure to increased temperature is not limited to the inorganic world, and the living body as well as the dead may be temporarily or permanently altered in form by mechanical means. Purely physical or purely chemical changes, such as these, are of but little interest to the physiologist, while, on the other hand, those changes which are peculiar to the living organism, the possession of which differentiates it from non-living nature, and which it no longer shows when life has passed away from it, are of paramount physiological importance. To inquire into the nature of such changes and, as far as may be, to trace them to their ultimate physical and chemical causes are the special tasks of physiology. The ultimate aim of the science, in a word, is not merely to attempt the elucidation of the several changes individually, but also to arrive at a clear comprehension of their relation to each other and, if it be possible, to solve the problem of the nature of life itself. Neither in its general nor in its special aspect has this goal as yet been reached. Whether it ever will be reached is a question to which varied and equally dogmatic answers have been given, some optimistic, some pessimistic, yet none of them can be said to be founded on any secure basis of evidence. The attraction which science exerts on the mind of man lies, however, not so much in the rapid attainment of the goal aimed at as in the actual scientific inquiry itself, and that is the reason why those who cry 'ignorabimus' have not long ere this abandoned scientific research altogether.

On inquiry we find that the changes which are characteristic of the living organism are as follows:—

1. Much the most easily observed are the changes in form which every organism exhibits during its life. From minute and for the most part simple beginnings, each organism gradually increases in size in obedience to certain recognized laws; it increases in complexity also, or, in other words, undergoes development, finally giving rise once more to the rudiments of a new organism, which in its turn passes through a similar developmental history. To these changes we shall give the name of **Metamorphosis**.

2. Changes of position, or movement of the entire organism or of its parts, are not readily observable in all forms. We shall find, however, that all organisms do exhibit such movements, possible only in consequence of

the expenditure of a calculable amount of mechanical energy. Moreover, we shall discover that organisms expend energies other than mechanical, such for example as heat, light, and electricity. These energies must have entered the organism in some form or another and must have undergone transformation within it. It follows that, in the organism, we meet with **Transformation of energy**.

3. The third series of changes are those which are so familiar in the animal world, viz. the absorption of materials from the external world, their transformation within the organism and the excretion of certain of these transformed bodies, that is to say, the changes summed up in the term **Metabolism**. In plants, also, a similar metabolism is observable, although special expedients are sometimes requisite to demonstrate its occurrence.

In the following pages we have to treat of transformation of form, of energy, and of materials, and we may most conveniently commence our studies by considering the last of these, transformation of materials or *metabolism*. A discussion of the physiology of organisms in general is outside our present task; we will confine our attention to the physiology of plants only. At the same time it must be borne in mind that the distinctions once believed to exist between the physiology of plants and the physiology of animals have become more and more obliterated, and that it has already become possible to elaborate a *general physiology* ([BERNARD, Leçons sur les phénomènes de la vie communes aux animaux et végétaux. Paris, 1878-9] VERWORN, 1894).

Before we enter on the discussion of the first great division of our subject, a few words on the **methods** of plant physiology will not be out of place. These methods are identical with, or at least do not differ in any essential particular from, those of physics and chemistry. In the first place, *observation*, and that too of the most exact character, is necessary for the proper study of the changes occurring in the organism; but observation alone is insufficient for the accurate determination of the *causes* of these changes. Plant life, as we shall discover, is maintained only in the presence of a whole complex of conditions, and it is only rarely possible to carry out a physiological observation under conditions of such a nature that we can say with confidence that a certain change takes place in the plant when, and only when, accompanied by a *single* change in the environment; then only can it be said that the special alteration in the surroundings is the cause of the special phenomenon in the plant. We have to employ the utmost care in contriving that only one of the many factors which affect the plant is altered. Observations made under such conditions are termed *experiments*. Owing to the nature of the case, physiological experiments are generally restricted within narrower limits than those of physics and chemistry. A purely physical experiment in plant physiology has for that very reason not infrequently led to most serious error, as an example will show. If a physicist were to fasten a wire by one end to some fixed point in a vertical position, and attach a metallic knob to the other, he might reasonably deduce that the wire bent over in consequence of the weight of the knob, and his deduction would be confirmed if, on removal of the knob, the wire once more became straight. A similar bending is seen in the peduncle of the flower-bud of the poppy, and we might very well conclude that curvature there also was due to gravity acting on the bud. If the bud be cut off, as in the case of the knob in the physical experiment above described, the peduncle, it is true, becomes straight, so we naturally conclude that the bud actually pulls the passive peduncle downwards. VÖCHTING (1882), however, has shown that when the weight of the bud is upheld the peduncle bends all the same, and that this curvature is maintained even when the pull from above more than compensates the weight of the bud. A consideration of these facts leads us to the conclusion that the weight of the bud has nothing to do with

the bending of the peduncle and that if we remove the bud the lesion itself induces the straightening of the peduncle. This experiment also teaches us to be on our guard against introducing, during the course of a physiological experiment, any new factor likely to produce special reactions on its own account.

A discussion of plant metabolism presupposes a knowledge of the chemical composition of the plant, and to this subject we must therefore first of all turn our attention.

Qualitative analysis discloses the existence in the plant body of a relatively small number of elements. Ignoring such as are found only in certain plants, such as are present only when artificially supplied, such as occur only in minute quantities, and which are obviously of no importance to the plant, there are left thirteen elements for consideration, viz. hydrogen, oxygen, chlorine, sulphur, nitrogen, phosphorus, silicon, carbon, potassium, sodium, calcium, magnesium, and iron. The gain in scientific insight which such an analysis affords us is, however, very limited.

Nor does *quantitative analysis* give us a much deeper insight into the subject, although the following table, borrowed from EBERMAYER (1882, p. 47), may prove useful as indicating the amount of carbon, hydrogen, oxygen, nitrogen, and mineral matter present in 100 parts of plant substance, dried at a temperature of 100° C. :—

	C.	H.	O.	N.	Ash.
Wheat grains . . .	46·1	5·8	43·4	2·3	2·4
Oats	*50·7*	*6·4*	36·7	2·2	4·0
Rye straw	49·9	5·6	40·6	*0·3*	3·6
Potatoes	44·0	5·8	*44·7*	1·5	4·0
Peas	46·5	6·2	40·0	4·2	3·1
Leaves of Red Beet .	38·1	5·1	30·8	*4·5*	*21·5*

On the other hand, information as to the *chemical compounds* occurring in plants is of much greater value. The number of these is, however, so enormous that it is out of the question to attempt an exhaustive enumeration of them ; moreover, investigations as to their nature are as yet far from being complete. We know, for example, of the existence of one or more compounds in certain species only, while there are many which appear to be characteristic of genera or families. The majority of these substances are *by-products* of metabolism and have on that account scarcely received from physiologists the attention they deserve. Putting on one side the inorganic compounds for the most part absorbed from the environment, and also the organic substances above mentioned which are of limited distribution, we have left for consideration a large number of organic compounds common to all plants. These are compounds of carbon with one or more of the elements, hydrogen, oxygen, nitrogen, sulphur, and phosphorus. It will be convenient at this point to present a brief summary of the more important of these bodies, classifying them rather according to their physiological value than their chemical constitution. It is impossible at the present stage to enter into a discussion of their chemical characters, although later on it may be necessary in special cases to give some details on these points. For all general questions of chemical constitution and peculiarities we must refer to the standard text-books on Chemistry and on Physiological Chemistry, such as those of EBERMAYER (1882), HAMMERSTEN (1899), and FÜRTH (1903). [Special reference must also be made to the recently published treatise by CZAPEK (Biochemie der Pflanzen, Jena, 1905. 2 vols.). It deals in a remarkably thorough manner with all the more important chemical problems in Plant Physiology and constitutes a handbook quite indispensable to the student of Biology.]

We may distinguish :—

1. **Organic acids.** Many of these by their very names show that they were primarily discovered in plants, e.g. oxalic, malic, tartaric, and citric acids, although they are by no means confined to the species whence they derive their names. The lower members of the fatty acid series are also very frequently met with in plants, e.g. formic, acetic, propionic, and butyric acids.

2. The **glycerides** of the **higher fatty acids** are known as **fats**, especially the glycerides of palmitic, stearic, and oleic acids. *Suberin* also is a glycerine compound of a fatty acid (suberic acid) and may for that reason be included here. Again the various *vegetable waxes* belong to the same category, for most of them are true fats or glycerine esters, some, however, are esters of univalent alcohols with fatty acids. Finally we may add *lecithin* and *cholesterin*, which have many characters in common with fats, but which have a more complex composition.

3. Among the **carbohydrates** we may note first of all the *monosaccharides*, which contain six carbon atoms (hexoses), such as glucose (dextrose), mannose, galactose, and levulose, or only five (pentoses), such as xylose and arabinose. The *disaccharides* have a larger molecular composition, and these bodies, by taking up water, hydrolyse easily into two hexose molecules ; e.g. cane-sugar decomposes into dextrose and levulose, milk-sugar into dextrose and galactose, maltose into two molecules of dextrose. The largest molecule occurs in the *polysaccharides* (starch, cellulose, &c.), which can be decomposed into several hexose and even pentose molecules.

4. **Amido-compounds**, i.e. amido-acids and acid amides. The *amido-acids* are derived from fatty acids by the substitution of NH_2 for H ; e.g. aspartic acid = amido-succinic acid ; leucin = amido-caproic acid ; alanin = amido-propionic acid, occurring especially in conjunction with phenol to form tyrosin. The *acid-amides* arise by substitution of NH_2 for OH in carboxyl ; e.g. asparagin = amido-succinic-acid-amide ; glutamin = glutaminic-acid-amide.

5. **Etherial oils** are familiarly recognized as the oily, volatile substances which are the source of many vegetable perfumes. From the chemical point of view we may distinguish (*a*) the *terpins*, simple hydrocarbons, e.g. of oil of turpentine and the oils which occur in the Myrtaceae and Umbelliferae ; to which group belong also caoutchouc and its relative guttapercha, the latter differing from the former, however, in having oxygen in its composition ; (*b*) *oxygen-containing bodies*, such as camphor and many of the oils of the Labiatae ; (*c*) *etherial oils containing sulphur*, such as those of certain species of *Allium* and of the Cruciferae.

6. The **resins** are related to the etherial oils, in which, as a matter of fact, they are not infrequently dissolved ; these bodies are chemically difficult to determine (TSCHIRCH, 1900).

7. The **alkaloids** are nitrogenous plant bases, familiar to us owing to the fact that to them may be attributed the poisonous properties possessed by very many plants. Their physiological significance is as yet but little known.

8. The **glucosides** are readily distinguished by the ease with which they can be decomposed into hexoses and various aromatic substances. Thus the nitrogenous substance amygdalin, found in bitter almonds, decomposes into glucose, oil of bitter almonds and hydrocyanic acid, the non-nitrogenous salicin gives saligenin and glucose. Many *tanning materials* also are related to the glucosides and yield by decomposition, in addition to gallic acid, a sugar or the 'aromatic sugar' phloroglucin. These substances concern us, as physiologists, but slightly.

9. The **pigments** in the plant are both chemically and physiologically extremely varied. We need only note here *chlorophyll* as the most important of them.

10. The **proteids** are at once the most important and also the most complex

constituents of the plant; they consist of carbon, hydrogen, nitrogen, oxygen, sulphur, and possibly also phosphorus.

Only a few examples of *quantitative analyses* of entire plants or of their larger parts are available. Such analyses have been made mainly in connexion with nutritive media, and are not of special interest at the moment, since they take into account substances belonging to too few groups. Still we may quote in this relation a short table illustrating such an analysis taken from KÖNIG (1882).

Percentage composition of fresh material.

	I	II	III	Non-nitrogenous.			IV	V	VI
	Water.	Nitrogenous material.	Fat. Ether extract.	Sugar.	Dextrine.	Starch.	Total.	Wood fibre.	Ash.
Wheat (grain)	13·65	12·35	1·75	(1·44)	(2·38)	(64·09)	67·91	2·53	1·81
Rye	15·06	11·52	1·79	—	—	—	67·81	2·01	1·81
Vicia faba	14·76	24·27	1·61	—	—	—	49·01	7·09	3·26
Yellow Lupin (seeds)	12·88	36·52	4·92	—	—	—	27·60	14·04	4·04
Coconut[1]	5·81	8·88	67·00	—	—	—	12·44	4·06	1·81
Potato tubers	75·48	1·95	0·15	—	—	—	20·69	0·75	0·98
Beetroot	87·71	1·09	0·11	6·53	—	2·73	9·26	0·98	0·95
Leek (leaves)	90·82	2·10	0·44	0·81	—	3·74	4·55	1·27	0·82
Lettuce (leaves)	94·33	1·41	0·31	—	—	—	2·19	0·73	1·03

[1] This estimate is taken from WIESNER, Rohstoffe des Pflanzenreiches, 2nd ed.

A few remarks may not be out of place with regard to the details of this table (comp. KÖNIG, 1897). In the first column the proportion of water present is indicated, from which it will be apparent that every part of the plant contains water, and that even in air-dried seeds it amounts to from 12 per cent. to 15 per cent. of the original weight, while plants in the living condition contain at least 75 per cent. of water, and usually, as a matter of fact, considerably in excess of that amount. The maximum percentage of water, viz. 98 per cent., occurs, as might be expected, in aquatic plants (Algae). Similarly, the last column teaches us that from no plant are mineral matters entirely wanting. Neither of these columns, so far as analysis is concerned, presents any difficulty, and both are of great physiological value. The case is quite otherwise with columns II, III, IV, and V. In order to arrive at an estimate of the nitrogenous constituents of the plant, the nitrogen itself was determined, and the number so obtained was multiplied by 6·25, because it was assumed in the first place that nitrogen occurred only in proteid, and, in the second place, that proteid contained 16 per cent. of nitrogen (N.B. $6{\cdot}25 \times 16 = 100$ per cent.). Neither assumption, however, proves to be correct. Proteid contains from 15 per cent. to 18·5 per cent. of nitrogen, and, further, nitrogen occurs in amides and in other bodies as well, probably in relatively large quantity. The information given in column II, therefore, is of limited value. Column III shows how much material is soluble in ether, all of which, however, is not fat; it includes as well such bodies as wax, lecithin, cholesterin, hydrocarbons, and chlorophyll. Column IV contains what is left over after subtracting the sum of the contents of the other columns from 100. In it are included by no means only carbohydrates, but everything soluble in dilute solutions of sulphuric acid and caustic potash (1·25 per cent. solution). The substances which resist such reagents are found in column V.

Quantitative analysis of a plant, though far more detailed and accurate than those given above, cannot give us any satisfactory insight into its chemical mechanism; for it must be obvious that materials which, in the process of analysis, become united in the distilling apparatus, occur in the

living plant localized in definite places, and cannot, under such circumstances, react in any way on each other. A glance through a microscope shows us how extraordinarily complicated in structure a plant really is. Every single organ is seen to be composed of innumerable units which we term cells, while, on the other hand, the entire body of a microscopic alga may consist of but one cell, similar to some one of those which occur in a higher plant. If we collect a number of these unicellular Algae and submit them, in mass, to chemical analysis, we shall obtain results in no respect differing from those which we meet with in a chemical analysis of the most highly differentiated parts of plants of much higher grade. It is manifestly of the highest importance that we should make ourselves acquainted with the constituent parts of a cell as revealed by the microscope, and ascertain, if possible, how the different substances which we have classified above are distributed in the cell. For this purpose we must employ not only the ordinary methods of chemical analysis, but also the so-called 'microchemical reactions', on whose further development the extension of our knowledge in this direction so largely depends. Even now, however, it is possible to determine *in situ* under the microscope a considerable number of chemical compounds. To enumerate all the microchemical reagents and their reactions would occupy far too much space; we must, therefore, content ourselves with a *résumé* of the most important results arrived at; in doing so, however, it will be impossible to avoid trenching on certain questions which are morphological rather than chemical.

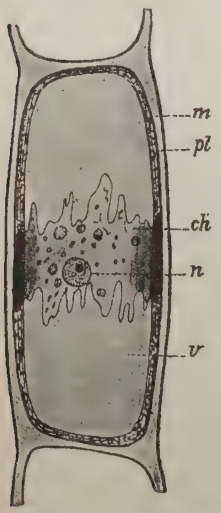


Fig. 1. Cell of *Draparnaldia glomerata*. *m*, cell-wall; *pl*, protoplasm; *ch*, chloroplast; *n*, nucleus; *v*, vacuole. (Magnified about 500.)

We may select as a subject for study a cell of the freshwater alga, *Draparnaldia glomerata*, illustrated at Fig. 1. This cell is cylindrical in form, and in it may be distinguished three primary constituents: (1) the *cell-wall* (*m*), which forms a hollow cylinder enclosing cell contents; (2) a soft, viscid substance, the *protoplasm* (*pl*), covering and closely applied to the inner surface of the cell-wall, and forming, like it, a closed sac; (3) the *cell-sap* (the *vacuole*, *v*), occupying the remainder of the space. Although no further structural differentiations are obvious in the cell-wall or cell-sap, these are by no means absent from the protoplasm. In the first place, we notice an annular green band with ragged edges, the *chloroplast* (*ch*), a spherical body known as the *nucleus* (*n*), and finally the *cytoplasm*, i. e. the remainder of the protoplasm, a colourless, translucent mass, whose exact nature it is extremely difficult to determine, and in which the chloroplast and the nucleus lie imbedded.

Structures of similar or nearly similar character are met with in the majority of vegetable cells, the difference lying for the most part in the form of the chloroplast. It is only rarely that that body has the characters seen in *Draparnaldia*, it is generally much simpler in structure; each cell may contain many chloroplasts, or these may be wanting altogether. The parts of the cell above enumerated are of very different value, for the functions fulfilled by the protoplasm are of much greater moment than those carried out by the cell-wall and cell-sap, the latter being really products of the former. Indeed, cells are not unknown which consist either for a time or during their entire life of protoplasm only. Protoplasm may be briefly defined as the *living substance* of the plant (and of the animal also), for it is only in such parts as contain protoplasm that we encounter those changes which we recognize as characteristic

of life. Obviously, then, a knowledge of the chemical nature of protoplasm will be of primary importance to us, but first of all, we may take note of some of the more important points in the chemistry of the cell-wall and cell-sap.

To start with, the *cell-wall* is obviously not a chemical entity, for in addition to the carbohydrates, of which it is in the main composed, it contains mineral matters and water as well. The *carbohydrates* which enter into its composition are polysaccharides belonging to the cellulose group. It is rarely the case that the cell-wall is composed of a single chemical compound; far more commonly many closely related bodies enter into its composition. The *water*, which is always present, is not, however, located in definite spaces in the wall, but occurs as 'water of imbibition', that is to say, in a state of minute subdivision between or within the ultimate molecules of the substance of the cell-wall itself. This is not the appropriate place to enter into the study of the subject of imbibition as a whole (Lecture XXXII), but we may note the following points of interest. In the first place, no definite and stable chemical union exists between the imbibed water and the substance of the cell-wall, since it is possible to squeeze the water out, at least in part, by mere mechanical pressure, or to permit it to evaporate into the atmosphere. What is still retained in the wall after pressure or evaporation may be driven off by heat. If the desiccated cell-wall be once more brought into contact with water, it absorbs it again with considerable force, and in quantity dependent directly on temperature. Simultaneously with the absorption of the water the volume of the wall is increased, and an important physical alteration takes place also in the swollen mass. Just as a piece of glue, hard and brittle as it is in the dry condition, becomes soft and pliable when wet, the cell-wall alters in character when similarly treated. For example, a fresh stem of *Cobaea scandens* (a well-known climber) can be twisted round one's finger like a piece of string, but if dried it becomes as brittle as glass. The alteration effected in the character of the cell-wall by the imbibition of water cannot be other than of the greatest significance in plant economy.

The *mineral matters* occurring in the wall may be in part dissolved in the water of imbibition, but most of them are distributed in the solid form in a minute state of subdivision among the particles of carbohydrate.

The *cell-sap* consists for the most part of water, in which are always dissolved a large number of organic and inorganic compounds, while there are also present in addition solid particles in suspension, resulting from the precipitation of substances normally soluble in the sap.

Microscopic investigation of the *protoplasm* reveals the presence of a hyaline ground substance, in which are imbedded the special organs of the protoplasm already referred to under the names of chloroplast and nucleus, as well as a number of granules and vesicles (microsomata), some of known, some of unknown composition. The varied streaming movements exhibited by this ground substance, or hyaloplasm, shows that it contains much water; indeed, it is easy to demonstrate directly its presence in the protoplasm. We may further assume that the relationship of the water and the protoplasm is similar in character to the relation subsisting between the water and the cell-wall; in other words, the protoplasm may be regarded as a swollen body. So far then as the hyaloplasm itself is concerned, we may consider it, when saturated with water, as a complex of organic nitrogen- and sulphur-containing bodies, or proteids, and looked at from this point of view it has often been the custom to regard protoplasm as proteid in solution. Because the cell-sap may also contain dissolved proteid, and because this proteid when removed from the plant no longer exhibits that vitality which makes protoplasm so interesting, a distinction was made between dead and living proteid, and the name protoplasm was confined to the latter. The chemical investigation of as pure

a sample of protoplasm as could be obtained (such as that carried out by REINKE and RODEWALD, 1881–3), was therefore of the greatest value. These investigators selected for the purpose the plasmodium of one of the Myxomycetes, viz. a mass of naked protoplasm, unenclosed by a cell-wall. Three quarters of this plasmodium was found to consist of water. The substance, when air-dried, gave on analysis about 5 per cent. of water and 28 per cent. of calcium carbonate. Since neither of these bodies can be considered as the specific medium of vital manifestations we may neglect them altogether. In addition, the air-dried plasmodium contained a large number of chemical compounds which could be estimated only approximately, and which did not lend themselves to accurate analytical determination. These estimates are summarized in the following table (REINKE, 1901. 232) :—

1.	Proteids containing phosphorus (plastin and nuclein)	about	40·0%
2.	Proteids not containing phosphorus	,,	15·0%
3.	Amide bodies	,,	1·5%
4.	Fats	,,	12·0%
5.	Lecithin	,,	0·3%
6.	Cholesterin	,,	2·0%
7.	Carbohydrates	,,	12·0%
8.	Resin	,,	1·5%
9.	Salts of organic and inorganic acids	,,	7·0%
10.	Undetermined or not specified bodies	,,	8·7%
			100·0%

We have already drawn attention to the fact that a series of metabolic processes takes place in the plant, consisting in the absorption of materials from the environment, the alteration of these in the plant, and an excretion of certain substances which have become of no further use; to this we must now add a further statement, viz. that these metabolic processes are primarily associated with the protoplasm. Chemical analysis of the protoplasm of the Myxomycetes does not give us any indication which of the compounds so determined form part of the special living protoplasm and which are to be considered as products of metabolism. REINKE expressly states that the plasmodia which he investigated were in the act of forming fructifications, and therefore were not likely to contain any unaltered nutritive materials absorbed from the environment. Since, however, the proteid, fat, and carbohydrate, known to occur in large quantities in the seeds of the higher plants, cannot be considered as genuine protoplasm, but rather as dead material deposited in these situations as plasta for the construction of the seedling, we are bound to regard the greater part, it may be, of the materials determined by REINKE as belonging to the category of so-called reserve materials. Again, it would be quite an arbitrary proceeding to designate any of the substances mentioned in the table quoted above (such as, e.g., the dominant proteids which contain phosphorus) as the essential, still less as the only constituents of protoplasm itself. It is possible that protoplasm, the true living substance, consists essentially of a mixture of many materials; there can, however, be only one substance with vital characteristics, and there is considerable evidence forthcoming, as we shall discover later on, tending to show that it is not unlikely that this substance exists only in relatively small quantity.

A further problem now confronts us. Is life bound up with one definite substance, which we may term 'the life-bearer', or does life arise from some special arrangement of lifeless bodies? It has often been the habit to liken the organism to a machine, and analogies between them are not wanting. But the work of a machine depends, in the first instance, not on the chemical nature of the substance of which its parts are composed, but on their mode of construction and on their arrangement. Whether we make a machine of brass

or of steel may well affect the durability and precision of the instrument, but not the nature of its action. Similarly one cannot help assuming that the mode of arrangement of the ultimate parts of the organism is of greater importance than the chemical nature of these parts.

Ernst Brücke (1861) deserves the credit of having been the first to lay stress on the more minute structure of protoplasm as the cause of its vital manifestations. He wrote in 1861 (p. 386), 'we are well acquainted with the fact that the structure of the molecules of the organic substances which enter into the composition of the cell is undoubtedly of a very complicated nature. But we cannot rest content with simply postulating a complicated molecular structure for the cell. It is impossible to think of a living vegetative cell as consisting of merely a homogeneous nucleus . . . and a proteid solution, for we certainly cannot perceive the phenomena which we term vital in the proteid as such. On the contrary we are forced to ascribe to the living cell an entirely distinct complexity of structure, quite apart from the molecular structure of the organic compounds of which it is composed, and to this complexity we may apply the term '**organization**'.

This organization demands special study, and for its investigation the newest and strongest objectives must be employed. As, however, A. Fischer (1899) has shown, investigations of this kind are certainly open to the possibility of serious error, because those who aim at conducting research on protoplasm are compelled, from the nature of the case, to operate upon dead protoplasm; since although great care be taken to preserve the natural structure in killing and fixing the material, still very frequently artificially produced precipitates have been mistaken for genuine features of the protoplasm.

According to many observers, protoplasm is composed of minute granules, according to others it is made up of threads or netlike aggregations of fibrillae, but all these theories of the structure of protoplasm are subject to the criticism that they are based on an examination of dead and stained protoplasm and not of the living substance. Bütschli's (1892) alveolar theory of the structure of protoplasm is free from this criticism, for undoubtedly there are many cases where a structure may be seen in living protoplasm suggestive of that exhibited by frothy liquids. [According to Fischer (Roux's Archiv f. Entwicklungsmechanik, Vol. 13, 1902) and especially A. Degen (Bot. Ztg. Vol. 63, 163, 1905), a foam-like structure in protoplasm is to be considered as a pathological phenomenon.] But whether only the walls of the honeycomb-like spaces, lying as they do at the limits of visibility, or their contents as well are to be considered as protoplasm it is impossible to say. It is equally certain that in other cases no such alveolar structure can be determined in *living* protoplasm, and thus we are forced to accept the views of Berthold (1886) and Fischer (1899) as most in accordance with our present knowledge, viz. that the constitution of protoplasm is neither uniform nor constant. Even if an alveolar structure, in the sense understood by Bütschli, could be demonstrated as of general occurrence in protoplasm, we should not gain much thereby, for Bütschli (1898) himself has shown that an alveolar structure may also be demonstrated in dead bodies and so cannot be regarded as a definite characteristic of living structure.

Up to the present, then, the organization of protoplasm has not been unravelled, although the investigations which have been carried out in this relation have done much to render clearer our conception of what protoplasm really is. No one nowadays doubts that it has a very complex structure, and we know that it cannot any longer be considered as a homogeneous solution. Further, no one expects to meet with the same or even an analogous structure in protoplasm as in a machine, the comparison of protoplasm with which must manifestly not be pressed too far. Owing to the manifold nature of the work

accomplished, and to the predominance of the chemical over the mechanical processes taking place in protoplasm, it is perhaps on the whole more appropriate to institute a comparison between protoplasm and a *chemical* laboratory or manufactory. In such a manufactory many chemical operations are carried out, possibly in the same room ; but many of these operations must be kept entirely apart if the wished-for result is to be attained. So also within a single cell both oxidation and reduction, anabolism and katabolism of protoplasm may take place, and for this reason alone *organization* in the protoplasmic body is essential, since conflicting operations must be kept isolated. F. HOFMEISTER (1901) has drawn attention to the fact that, looked at from this point of view, an alveolar structure in the protoplasm may have a deep significance. Every one of the countless cavities might be considered as a chamber cut off temporarily or permanently from the exterior. Within the space of a cubic μ the most heterogeneous reactions may take place. The aim of future research must be, without over emphasizing either the material of which protoplasm is composed or its organization, to take both of these phenomena into consideration, and to employ them as bases for wider research in this most difficult subject. At the same time it may be expressly noted that purely chemical research into the nature of protoplasm is by no means valueless, and that it has not yet been shown that chemical peculiarities play no part in protoplasmic phenomena. It is obvious that we have not as yet discovered any living chemical compound or any mixture of chemical compounds which may be considered as vitally active, since the physiological chemist must of necessity kill the very living substance he desires to study in subjecting it to analysis. Experiments in physiological chemistry have shown sufficiently that in proteids we are dealing with substances not only exceedingly complex but also very delicate and labile, substances which may become continually altered owing to apparently quite trifling causes. One can scarcely doubt that the transformations which are exhibited by protoplasm, e. g. through the influence of gentle pressure, and which result in local or general death, depend not merely on changes in organization but also on chemical changes which are irreparable. Thus chemical alterations are set up if water enters a protoplasmic particle previously surrounded by salt solution, and these alterations may be expected to be even more pronounced when the contents of two alveoli or two vacuoles, originally separate, become united. REINKE (1901) says that 'if one gently bruises in a mortar some pure protoplasmic rudiments of fruits of Mycetozoa, the substance remains unaltered quantitatively and, to all appearance, also chemically, but its organization is irretrievably destroyed, so that subsequent differentiation of fruits is completely inhibited ; in other words, the protoplasm is killed by the pestle without chemical action of any sort'. This view we cannot agree with, for although the rubbing in the mortar may not, it is true, have affected the substances enumerated in the analysis given above, still the albumins and phosphorus-containing proteids must have been greatly modified.

The data we have obtained as to the chemical composition and structure of protoplasm are applicable not only to protoplasm as a whole, but individually also to cytoplasm, nucleoplasm, and chloroplasts. Since the nucleus and chromatophores are living parts of the plant they may be considered as organs of the protoplasm. From the chemical standpoint there are many agreements as well as many differences between the cytoplasm and the nucleoplasm, but we need not follow out these in further detail at this stage, since they have not as yet been shown to have any general physiological significance. For the same reason we may omit here any reference to the results obtained from investigations into the chemistry of the proteids. It is to be hoped, however, that these results may soon prove to be of greater physiological value than heretofore.

To summarize, therefore, it may be said that our first attempt to obtain

an insight into the chemical architecture of the plant has brought us face to face with questions of the most difficult character, questions which we must leave without being able to give satisfactory answers to them. At the same time, chemical investigation of a selected plant teaches us much that is of importance. In the first place, we find the plant to be composed of elements all of which are found in its surroundings, the soil, water, and air ; and, in the second place, we see that these elements are grouped in the plant into more complex compounds than occur in the inorganic environment. We have also seen that the plant takes up its raw materials from this environment, and changes them in its interior. We must now endeavour to make ourselves acquainted more in detail with the absorption of these raw materials.

Bibliography to Lecture I.

Berthold, G. 1886. Studien über Protoplasmamechanik. Leipzig.
Brücke, E. 1861. Die Elementarorganismen. Sitzber. Wien. Akad., Mat.-nat. Kl. 44, Abt. ii, p. 381 (Ostwald's Klassiker, Nr. 95, Leipzig 1898).
Bütschli. 1882. Unters. über die mikrosk. Schäume u. d. Protoplasma. Leipzig.
Bütschli. 1898. Unters. über Strukturen, etc. Leipzig.
Ebermayer. 1882. Physiologische Chemie d. Pflanzen, I. Berlin.
Fischer, A. 1899. Fixierung, Färbung und Bau des Protoplasmas. Jena.
Fürth, R. v. 1903. Vgl. chemische Physiologie d. niederen Tiere. Jena.
Hammarsten. 1899. Lehrbuch der physiologischen Chemie, 4th ed. Wiesbaden.
Hofmeister, F. 1901. Die chemische Organisation der Zelle. Braunschweig.
König. 1882. Zusammensetzung der menschlichen Nahrungs- und Genussmittel, 2nd ed., Vol. i. Berlin 1882.
König. 1887. Landwirtsch. Versuchsstationen, 48, 81.
Reinke and Rodewald. 1881. Unters. aus d. bot. Lab. Göttingen, 2, 1.
Reinke. 1881. Ibid. 2, 79.
Reinke. 1883. Ibid. 3, 1.
Reinke. 1901. Einleitung in die theoretische Biologie. Berlin.
Tschirch. 1900. Die Harze und die Harzbehälter. Berlin.
Verworn, M. 1894. Allgemeine Physiologie, 3rd ed. Jena 1901.
Vöchting, H. 1882. Die Bewegungen der Blüten und Früchte. Bonn.

LECTURE II

THE OSMOTIC CHARACTERS OF THE CELL

The mode of absorption of nutritive materials may be best studied by a consideration of the process as it occurs in a single cell. If the cell be naked, as it is, for example, in the Myxomycetes, the protoplasm can flow round and absorb solid bodies ; but in the great majority of cases the existence of a rigid cell-wall renders the absorption of solids impossible and compels the plant to be dependent on fluid nutriment, and absorption of water and of substances dissolved in it is almost the only method available in nature by which the plant can obtain its food supply. That water can penetrate both cell-wall and protoplasm is unquestionable, since, as we have already seen, both are capable of swelling in water ; but whether the substances dissolved in the water are also capable of entering is quite another question. As a matter of fact, we shall find that by no means all substances soluble in water are able to enter the cell. When we inquire whether this selective absorption depends on the nature of the cell-wall or of the protoplasm we find ourselves compelled to study the physical laws of diffusion and osmosis, for it is in this category of phenomena that we must include the processes concerned in the absorption of nutritive material by the cell.

It is well known that when two miscible liquids or solutions, e. g. alcohol and water, or water and an aqueous solution of copper sulphate, are care-

fully poured consecutively into a glass cylinder, they will remain at first distinct from each other. Sooner or later, however, the sharp boundary between them vanishes, as the copper sulphate molecules begin to wander in amongst the water molecules and vice versa. This phenomenon is known as diffusion, and it continues until the intermixture of the two fluids is complete, and until the whole becomes uniform in character and in concentration. Suppose, however, that the two fluids have no free surfaces but are separated by a porous wall, then the phenomenon takes place under altered conditions, and is known as osmosis. Let us assume that the fluids are water and an aqueous solution of copper sulphate, and, further, that these fluids are placed in the legs of a U-tube, separated by an intervening wall of clay (*s*, Fig. 2), animal bladder, or parchment; it will be found that the two fluids do not pass through the dividing wall with equal rapidity; the water, as a matter of fact, passes far more rapidly into the copper sulphate than the copper sulphate solution into the water. The result is obviously an increase of fluid on the copper sulphate side, the level of the fluid on that side rising in proportion as it falls on the other. Similar results are obtained when copper sulphate is replaced by selected salts or by alcohol. That the result is essentially dependent on the nature of the separating wall is shown by the fact that when a thin caoutchouc membrane is introduced between alcohol and water, more alcohol passes through the membrane into the water than water into the alcohol. In every case, however, so long as the separating membrane is permeable to both bodies, a complete intermixture in the long run always takes place, resulting in the same concentration of fluid on both sides of the dividing wall.

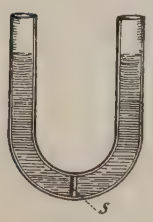


Fig. 2.

The cell-wall comports itself precisely like the parchment or animal bladder in this experiment, presenting less resistance to the passage of water than of salt solutions. In course of time the salts also manage to pass through, and it is only to chemical compounds with large molecules, like gum, proteid, &c., that the wall is quite, or almost quite, impermeable.

Protoplasm in its osmotic characters differs from the cell-wall and agrees with the so-called semipermeable membrane in one very important point, viz. that it is quite impermeable to certain materials, such as many salts, sugar, &c., although it is readily permeable to water. If one places such a semipermeable membrane in the position of the dividing wall in the experiment just described (Fig. 2) the water passes into the copper sulphate, but no trace of the copper sulphate passes into the water. Under these conditions a uniform mixture of the two fluids, resulting in similar concentration on either side of the membrane, cannot take place; one substance must always be in excess on one side of the dividing wall. As an example of a semipermeable membrane we may take the so-called 'precipitation membranes' produced by the contact of aqueous solutions of ferrocyanide of potassium and copper sulphate or of tannin and gelatine. The precipitation membranes thus obtained, gelatine-tannate or copper-ferrocyanide, do not form suitable experimental materials, nor can they be introduced conveniently into the U-tube shown at Fig. 2. Even were their introduction possible, these membranes are so fragile that they would be quite unsuitable for the purpose. For those reasons PFEFFER (1877), in his fundamental researches on this subject, precipitated the copper-ferrocyanide membrane in or on the wall of a porous pot, such as is used in a Daniell's element. The precipitation membrane is thus supported, and the apparatus takes the form represented at Fig. 3. If the porous pot be filled with a 10 per cent. solution of cane sugar to which the copper-ferrocyanide membrane is impermeable, and if the pot be then placed

in water, the latter at once begins to enter through the wall, and the diluted sugar solution begins to rise in the tube (*R*). If the U-tube be now filled with mercury (*Qu*) it will be found that the water enters with a force sufficient to raise and support a column of mercury of considerable height. This pressure is usually explained by postulating an attraction between the molecules of sugar in the porous pot and the molecules of water outside. The attraction, however, affects the water only, for the sugar does not pass out on account of the precipitation membrane being impermeable to that substance. The amount of the attractive force may be estimated, starting from the position of rest, by measuring the height of the mercury column which is supported by the inflowing water.

PFEFFER'S osmotic cell may be legitimately compared with a vegetable cell, especially if the precipitation membrane be laid down on the inside of the porous pot, as shown in Fig. 3, as is possible for many experiments without detriment to the rigidity of the membrane. The cell-sap (which itself may contain cane sugar) would correspond to the contents of the pot, the protoplasm to the copper-ferrocyanide membrane and the cell-wall to the wall of the pot. If the cell of an alga (Fig. 1) be placed in water, the water streams through the cell-wall and protoplasm into the vacuole, and if it were possible to attach a graduated manometer to this cell, the pressure exerted on the inside of the cell-wall might be estimated. That such a pressure actually exists may be easily demonstrated otherwise. The pressure of the inflowing water induces tension in the elastic cell-wall, and if this tension be relieved, e.g. by piercing the wall, a distinct contraction of the cell may, in many cases, be observed. This internal pressure is termed osmotic or turgor pressure, and by it the protoplasm is pressed firmly against the membrane; without such a resistant layer the protoplasm would have been as little able to resist this pressure as the copper-ferrocyanide membrane would without the supporting clay cell.

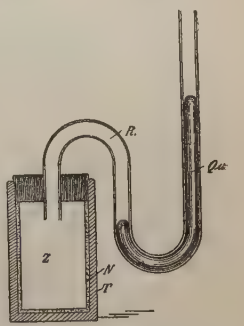


Fig. 3. PFEFFER'S osmotic cell. *T*, porous pot; *N*, precipitation membrane; *R*, manometer containing mercury, *Qu*; *Z*, sugar solution.

Since protoplasm, however, differs widely in consistence from a precipitation membrane made of gelatine-tannate or copper-ferrocyanide, and may be likened rather to a fluid than to a solid, it is important to note that genuine fluids also show the phenomenon of semipermeability. Water, for example, is permeable to ether, but not to benzol, and if a membrane be saturated with water and abuts on one side on pure ether, and on the other on benzol, all the conditions are fulfilled for the establishment of an excessive osmotic pressure on the benzol side (NERNST, 1890).

If we next ask ourselves what substances the protoplasm is permeable to and what not, two methods are open to us, either to determine those capable of diffusing outwards from the cell-sap to the exterior (exosmosis), or inwards from the environment into the vacuole (endosmosis). Our knowledge of the contents of the vacuole is, at least in some cases, sufficiently extensive to enable us to determine that in the long run exosmosis will take place from it. We know, for example, that the cells of sugar-beet are unusually rich in cane sugar, a substance which may, by chemical methods, be detected even in minute quantities. When thick slices of sugar-beet, thoroughly washed to remove all traces of free sugar released from the cut cells, were laid in water, DE VRIES (1877) found that even after fourteen days no sugar had passed from the uninjured cells into the water. Again, if this experiment be repeated, using red beet in place of white, we find that the protoplasm is as impermeable

to the colouring matter as to the cane sugar. In certain cells, however (nectaries, Lecture V; roothairs, Lecture VIII; endosperm cells, Lectures XIII, XIV), the protoplasm is found to be permeable to various substances, such as sugar, asparagin, peptone and proteid. In other cases we must admit the fact of exosmosis without being able to say definitely what the substances are which undergo exosmosis. Take, for example, Bacteria, which require organic compounds for their nourishment; if these organisms are able to thrive at the expense of green Algae it is manifest that exosmosis of organic materials must take place from the cells of the Algae. Again, the seeds of *Lathraea* and *Orobanche* germinate only in the presence of a host plant, and this fact cannot be explained otherwise than by assuming that an exosmosis of organic compounds takes place from the host. But in general it may be said, as shown by De Vries in the case of beet, that no exosmosis of the contents of the vacuole takes place.

A comprehensive knowledge of the phenomena connected with the permeability of protoplasm cannot, however, be obtained by a study of *exosmosis* only, because we are limited to the accidental occurrence of substances in the vacuole, our acquaintance with which, moreover, is only rarely exact. In studying *endosmosis*, on the other hand, we are unlimited in our choice of the materials which we may present to the cell; whether we obtain results or not will depend solely on our ability to establish a definite criterion for absorption or non-absorption. Such a criterion of protoplasmic impermeability, indeed the

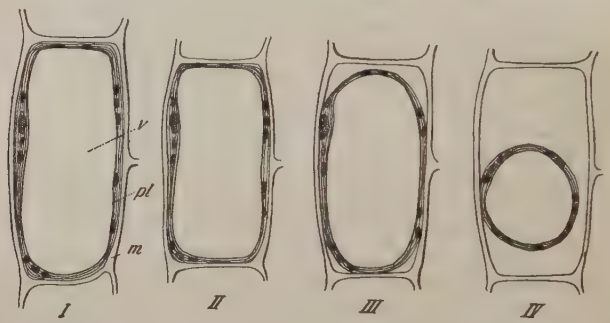


Fig. 4. Young cells from the cortical parenchyma of the peduncle of *Cephalaria leucantha*. *m*, cell-wall; *pl*, protoplasm; *v*, vacuole. *I*, in water; *II*, in a 4% solution of potassium nitrate; *III*, in a 6% solution of the same; *IV*, in a 10% solution of the same. (After De Vries, 1877.)

best one available, is *plasmolysis*, a phenomenon first enunciated by Nägeli (1855), and afterwards so thoroughly worked out by De Vries (1877) and Pfeffer (1877), that at the present day it is perhaps not only amongst the most fully investigated phenomena in plant physiology, but, far beyond the special branch of science concerned, has become a subject of the greatest interest to students of general chemistry.

Suppose we revert to our alga cell and assume that we know the actual contents of the fluid in the vacuole, that it contains a 10 per cent. solution of cane sugar; what happens when this cell is placed in pure water we have already seen, but what will happen if we place such a cell in a solution of cane sugar which has, let us say, a lower concentration than that presented by the cell-sap? From such a solution the vacuole will obviously withdraw less fluid than from pure water, less and less in fact as the fluid without becomes more and more concentrated. When the concentration of the fluid outside is equal to that of the fluid in the vacuole, no water will be taken up at all from the surroundings; when, on the other hand, the fluid outside has a higher concen-

tration than the contents of the vacuole, *water will be withdrawn from the vacuole, which in consequence will become smaller.* At this point the behaviour of the cell membrane differs from that of the protoplasmic lining, for the protoplasm is capable of contracting proportionally to the decrease in size of the vacuole, while the rigid cell-wall, on the other hand, can contract only to the extent of regaining the size it possessed before being elastically extended by the osmotic pressure. When the tension of the cell-wall is relaxed (Fig 4, *II*) no further decrease in size is possible, and a state of *plasmolysis* ensues, where the protoplasm becomes separated from the cell-wall. This separation appears first at the angles of the cell (Fig. 4, *III*) and proceeds continuously therefrom, until the whole of the protoplasm has assumed the form of an elliptical or spherical mass lying free in the interior of the cell (Fig. 4, *IV*). If the solution employed for bringing about plasmolysis be coloured by a suitable dye, e. g. indigo-carmine or aniline-blue, it will be seen that the coloured fluid penetrates the cell-wall and fills the space between the wall and protoplasm. Further, this experiment demonstrates the fact that the membrane is permeable to the dye, but that the protoplasm is not. If we once more immerse the plasmolysed cell in water, plasmolysis disappears and the cell regains its original size and form, without apparently having suffered any injury. On the death of the protoplasm, e. g. as a result of a sufficiently high temperature, its diosmotic characters are completely altered ; dead protoplasm presents no impediment to the free passage of colouring matters, salts, &c.

By employing the plasmolytic method of investigation we are able readily to determine that protoplasm is *impermeable* to a large number of substances even though these be soluble in water. If only the proper degree of concentration be ascertained, plasmolysis can be affected by cane sugar as well as by grape sugar, by common salt as well as by potassium nitrate, but the exact concentration must be determined by experiment. With the view of ascertaining the relative plasmolytic or osmotic activities of different substances it is necessary to determine the precise concentration of each which will bring about the first trace of plasmatic retraction from the wall (Fig. 4, *III*, lower part of the cell). The degree of concentration which induces this change may be considered as having a slightly higher osmotic value than that of the cell contents, while a concentration exactly similar to that of the cell contents will obviously produce no reaction at all. Thirty years ago, by the plasmolytic method, De Vries studied empirically the degrees of concentration of a variety of substances which were capable of giving equivalent osmotic effects (briefly termed *isosmotic concentrations*). The material he employed was red beet, and the results he obtained were as follows :—

1. Cane sugar	27–28%	5. Sodium nitrate	6–7%
2. Magnesium sulphate	26–28%	6. Potassium chloride	4–5%
3. Sodium sulphate	17–18%	7. Sodium chloride	3–4%
4. Potassium nitrate	6– 7%		

At first sight these numbers appear to follow no law, but in 1884, De Vries, after the examination of a very large number of variously constituted substances, successfully established a definite relation between the different isosmotic solutions. He was able to show, as indeed was only to be expected, that the osmotic effect depends not on the *specific gravity* of the substance but on the *number of the molecules* dissolved. Now if substances be dissolved in quantities proportional to their molecular weights, similar numbers of molecules will be obtained in each solution. When as many grams are dissolved in a litre of water as is indicated by the molecular weight of the substance we speak of this unit as a *gram-molecule to the litre* (briefly 'G.M.'). Thus to obtain a G.M. of cane sugar,

342 g. must be used ; a G.M. of KCl requires 74 g., a G.M. of NaCl requires 58 g., per litre in each case. De Vries also discovered that a G.M. of invert sugar, cane sugar, malic acid, tartaric acid, citric acid, &c.—in short, a G.M. of every non-metallic organic substance soluble in water had exactly the same osmotic value, whence we may deduce the law that *equimolecular solutions are at the same time isosmotic.* It is worthy of note that the plasmolytic method may also be employed in chemistry for the determination of molecular weights, as De Vries (1888 b) has shown in the case of raffinose. Various formulae have been put forward by different chemists for this sugar, each giving a different molecular weight :—

1. $C_{12}H_{22}O_{12} + 3H_2O$; M.W. = 396
2. $C_{18}H_{32}O_{16} + 5H_2O$; M.W. = 594
3. $C_{36}H_{64}O_{32} + 10H_2O$; M.W. = 1188.

If now the same material be treated with equivalent plasmolytic solutions of raffinose and cane sugar, we find that a 3·42 per cent. solution of cane sugar is isosmotic and equimolecular with a 5·957 per cent. solution of raffinose. But a 3·42 per cent. solution of cane sugar is the equivalent of 0·01 G.M. to the litre, therefore a 5·957 per cent. solution of raffinose must have a similar G.M., and its molecular weight must be 595·7, a number which strikingly resembles the second of the formulae quoted above. The correctness of this conclusion has been confirmed lately by other methods.

What is true of organic non-metallic substances is, however, far from being so of all compounds. We might expect a solution of 101 g. of potassium nitrate in 1000 g. of water to give exactly the same osmotic effect as 342 g. of cane sugar; in fact, it has almost the same effect as 1·5 G.M. of cane sugar, in other words, one and a half times as much as one would expect. De Vries (1884) has, moreover, shown that in the case of many substances one may obtain one and a half, two, or two and a half times the value of an equimolecular sugar solution. He assumed, to use round numbers, the value of a known sugar solution to be 2, and found that other substances gave, more or less exactly, the numbers 2, 3, 4, and 5. These numbers may be termed, following Pfeffer's nomenclature, *isosmotic co-efficients,* for they indicate how much greater the osmotic value of the substance is as compared with sugar = 2, and a knowledge of such co-efficients is of the greatest value in all plasmolytic research.

If we further bear in mind that these isosmotic co-efficients apply only to *dilute* solutions, the fact that a solution of, e. g., potassium nitrate has a value one and a half times as great as what we would expect from its molecular weight becomes at once clearly explicable. From a consideration of countless instances, modern chemistry has shown that in dilute solutions molecules in part dissociate into their constituent 'ions', each free ion having the same osmotic value as the entire molecule, i. e. it attracts water with the same force as the molecule does. The degree of dissociation depends on the one hand on the degree of dilution of the solution, and on the other on the nature of the dissolved substance. In the end all the molecules may become dissociated, and these isosmotic co-efficients give, not, it is true, an exact, but still an approximate, indication of the amount of dissociation which has taken place; their significance is purely practical and they are useful on the whole if we desire to calculate the osmotic value of a definite solution.

We have spoken above of a force with which the dissolved substance and the medium, water, attract each other. This conception was until recently generally accepted ; but nowadays, owing to the advance in general chemistry, it has come to be considered an old-fashioned. Osmotic pressures developed in cells are now explained by reference to certain characteristics exhibited by all bodies in solution. According to Van't Hoff's theory, one must ascribe to substances in solution the same characteristics as are ascribed

to gases, and since the kinetic theory of solutions and of osmotic pressure is based experimentally on PFEFFER'S researches in vegetable physiology, it will be appropriate to consider this theory briefly here.

In the course of his investigations on osmosis, PFEFFER, in 1877, demonstrated that the high osmotic pressures whose existence in certain vegetable cells had been proved by him a short time previously, were due to the presence of *crystallizable* bodies, such as cane sugar, potassium nitrate, and other substances with relatively small molecules, although, until that date, physicists had held that high osmotic pressures could be attained only by colloids, such as proteids, gums, &c., with *large* molecules. Previously to Pfeffer's experiments only such materials as parchment and animal bladder had been used as separating membranes in the osmometer, and with colloids these gave higher osmotic pressures than crystalloids, the reason being that the crystalloid diffused with great readiness. PFEFFER showed that by using semipermeable precipitation membranes instead of such materials, high pressures could easily be obtained with the aid of crystalloids. The artificial cell employed by PFEFFER was a porous pot in the wall of which a copper-ferrocyanide membrane had been laid down, as described above, and using sugar solutions of different concentrations he obtained the following pressures as indicated by heights in centimetres of mercury :—

Sugar in weight %.	Height in cm. due to pressure.
1	53·8
2	101·6
4	208·2
6	307·5
1	53·5

We may note, in the first place, that the osmotic activity which brings about the pressure is almost exactly proportional to the degree of concentration. An *average* of several experiments gives us 50·5 cm. of mercury for a 1 per cent. solution, and thus a 34·2 per cent. solution (= 1 G.M.) will give a pressure of 1,727 cm., or 22.7 atmospheres. This number forms the basis on which VAN'T HOFF founds his comparison of osmotic pressure with the pressure of gases. BOYLE'S law, governing the pressure of gases, states that the product of the volume and of the pressure of a gas is a constant quantity ; if the volume of the gas decreases, then the pressure must increase correspondingly. Under ordinary atmospheric pressure 1 G.M. of oxygen (32 g.) or 1 G.M. of carbon-dioxide (44 g.) occupies 22·4 litres. To compress the gas until the G.M. occupies only one litre will necessitate a pressure of 22·4 atmospheres. The pressure of 1 G.M. of gas in the litre corresponds exactly, as one would expect, to the pressure of 1 G.M. of cane sugar in PFEFFER'S osmotic cell, and on this correspondence is based VAN'T HOFF'S theory, i. e. that osmotic pressure arises from the impacts at the same time of the molecules dissolved in water and of the ions on the wall of the cell. However, botanical investigations on the amount of osmotic pressure are entirely independent of this physical theory.

As it is quite immaterial in the case of gases whether the separate molecules in the vessel are all of one kind, or are chemically different, so in considering the amount of osmotic pressure in the plant cell it is immaterial whether the cell-sap contains *only* cane sugar, as we have hitherto assumed, or whether it contains a complex mixture. So long as these bodies do not react on each other, so long as the number of molecules and ions remains unaltered, and so long as the protoplasm remains impermeable, so long does the osmotic pressure remain unaltered, and we can always estimate its amount by the plasmolytic method whether we know the nature of the materials which produce it or not. If we find that a 3·5 per cent. solution of cane sugar will bring about the first trace of plasmolysis in certain plant cells, while a 3 per cent. solution produces no effect, it follows that the cell-sap is approximately isosmotic with a 3·5 per cent. solution of cane sugar, and that the cell exercises the same

pressure on the cell-wall as the sugar solution would, viz. about two and a half atmospheres. We may, however, employ other substances to bring about plasmolysis, for so long as we know the molecular weights of these substances and their isosmotic co-efficients, the estimation of osmotic pressure by the plasmolytic method presents no difficulty. Potassium nitrate has often been used in place of cane sugar because it has been found that protoplasm is frequently quite impermeable to that salt. A 1 per cent. solution of potassium nitrate is equivalent to a 5·13 per cent. solution of cane sugar.

All vegetable cells are not equally suitable for plasmolytic research. *Young* growing cells have their membranes stretched owing to osmotic pressure, and such cells contract, as we have seen, when that pressure is withdrawn. This contraction must also take place in plasmolysis, and so complications in endeavouring to estimate osmotic pressure in such cells are introduced, which had best be avoided. But neither are all *mature* cells suitable for the purpose; often the first beginnings of plasmolysis are not readily observable, and yet that is the point of importance. For plasmolytic research in general, rather than the determination of osmotic pressure in specific cells, it is preferable to employ *mature cells* containing a *coloured cell-sap*, in which the separation of the protoplasm from the wall may be especially well seen. De Vries, for example, recommends the epidermal cells from the underside of the leaf of *Tradescantia discolor*, and this material is frequently used for this purpose.

This is not the place to quote detailed statistics as to the absolute amount of osmotic pressure (compare Lecture XXXIII); it will be sufficient to note that pressures of five to ten atmospheres are by no means infrequent in the plant. Deviations from such average pressures are known to occur, both above and below the mean. Osmotic pressure does not appear, however, to sink under three atmospheres in starved cells—whilst it may reach fifteen to twenty atmospheres in such plants as the beetroot and onion.

Young cells which contain no vacuoles also exhibit a turgor pressure. In this case the osmotically active substance must be dissolved in the protoplasm; later on, it preponderates in the vacuole, where it accumulates proportionally as the volume of the vacuole increases *pari passu* with the volume of the cell in the process of growth.

In the cells of the beet and the onion, which we have instanced as examples of plants showing especially high osmotic pressures, the turgor is manifestly due to the useless activity of the accumulated reserves. When these substances, as happens frequently in other regions of storage, are condensed into larger molecules, e. g. starch, and rendered *insoluble*, then the osmotic pressure disappears. In many other cells also it is possible that the accumulation of reserves may bring about a pressure capable of producing perhaps a quite undesirable secondary effect; but that is not of general occurrence. Osmotic pressure has frequently one very definite function to fulfil. By its means, as we have already seen, the cell membrane is kept tense, and so long as the tension is maintained the cell is more rigid than in the plasmolysed condition. Just as inflation of a caoutchouc bladder renders it rigid, owing to the stretching of the membrane, so osmotic pressure induces a corresponding rigidity in a plant cell. In thin-walled growing cells this rigidity is maintained *entirely* by osmotic pressure, and a small loss of water at once relaxes the tension of the cell-wall and annuls the rigidity of the cell. The condition of tension is termed turgescence and we speak of it as caused by turgor pressure; turgor pressure is identical with osmotic pressure. (As to the part played by turgor pressure in growth, see Lecture XXI.)

The net result of our discussion, looked at from the point of view of the *absorption of nutritive material*, is that we have found protoplasm to be easily permeable to water, but quite impermeable to many of the substances soluble in it. This result appears all the more surprising when we investigate how

the materials met with in cells enter into it. From the fact that water is not the only body found in the cell it follows of necessity that all materials do not behave in the way that cane sugar, potassium nitrate, and the colouring matter of red beet do; there must be many materials which are capable of passing through the protoplasm. The researches of later years have proved to us the existence of many such bodies, whose capacity for penetrating the protoplasm may be demonstrated in a great variety of ways.

The plasmolytic method is equally serviceable for the demonstration both of permeability and of impermeability. De Vries (1888 a) has shown that *glycerine* produces plasmolysis at first, but that after several hours this plasmolysis disappears, the reason for the disappearance being that glycerine begins slowly to enter into the interior of the cell. When the degree of concentration of the glycerine inside and outside the cell is the same, the turgor of the cell is re-established, and the existence of glycerine on *both sides* of the cell has as little significance in relation to turgor as if it were *entirely absent*. The osmotic pressure has, however, increased, and if the cell be once more plasmolysed it will be found necessary to use a more concentrated solution than before.

Similarly, plasmolysis produced by urea, erythrite, glycol, &c., ceases to make itself apparent, sometimes more rapidly, sometimes more slowly (Overton, 1895). Only a few minutes are necessary for the cessation of plasmolysis in *Spirogyra* when treated with glycol, acetamide, and succinamide, while plasmolysis, produced by glycerine, continues for a couple of hours, by urea five hours, and by erythrite twenty hours. Overton has, however, discovered that certain substances, e. g. alcohol, pass through the protoplasm more rapidly than glycol, and produce practically no plasmolysis, behaving in this respect like water. Since the molecular weight of alcohol is 46, a 1 per cent. solution of that substance has the same osmotic value as a 7·5 per cent. solution of cane sugar, so that, if an 8 per cent. solution of cane sugar produces plasmolysis in a cell of *Spirogyra*, the same effect should be obtained by using a 1·1 per cent. solution of alcohol. No plasmolysis is, however, observable on using either a 1 per cent., 2 per cent., or even 3 per cent. of alcohol, for it passes too rapidly through the protoplasm to give time for plasmolysis to take place. That this result is due really to the rapid penetration of the plasma, and not at all to injury induced by the alcohol, is shown by the fact that if the 3 per cent. solution of alcohol be added to the 8 per cent. solution of sugar, plasmolysis takes place at once just as when the sugar is dissolved in pure water. In a similar way Overton has established the fact that protoplasm is easily permeable to a large number of organic substances, such as ether, chloralhydrate, sulphonal, caffein, antipyrine, &c.

The fact that many substances enter into the cell in this manner is indisputable, but their entry is not perfectly *self-apparent*. We cannot actually see the entrance of the substance, we can only *conclude* that it enters. At least we must assume this from what has been said, although it does not quite correspond with the facts. Not a few of the substances referred to betray their entrance by the changes which are set up by them in the cell, such, for example, as the deposition of insoluble bodies in the cell-sap after the entry of caffein, antipyrine, acetamide, ammonium carbonate, &c. Generally speaking, we are quite ignorant of the nature of these precipitates, save that in certain cases, e.g. caffein and antipyrine, it is known that they are associated with the formation of insoluble tannin compounds. Tannin itself is soluble, but, since these tannin compounds are insoluble, the tannin is itself precipitated. In the case of ammonium carbonate, probably the alteration of the acid reaction of the cell-sap only plays a part. This can be proved with greater certainty when the cell-sap is naturally coloured (e.g. as in red beet and in red and blue flowers), the colouring matter serving as an indication of the presence of acids or bases, much in the same way as a solution of litmus would. Thus the entry of very

dilute solutions of free acids and of alkalis may be observed to take place without any injury ensuing to the protoplasm.

The penetration of protoplasm by aniline dyes, a knowledge of which we owe to PFEFFER (1886), is a phenomenon of great interest and importance. Since colouring matters naturally dissolved in the cell-sap are unable to undergo exosmosis, so long as the protoplasm is in a normal condition, the protoplasm has been generally regarded as impermeable to dyes; at the same time, we have long been aware that *dead* protoplasm can absorb and accumulate many pigments. The majority of the aniline dyes are poisonous to the cell, and unless very dilute solutions be employed, they kill the protoplasm, before they penetrate it. Among the relatively non-poisonous aniline dyes, methylene blue ranks first, for the plant can endure a solution of 1 in 100,000, or even 1 in 10,000, without suffering any injury. A solution of methylene blue of a strength of 1 in 100,000 exhibits a beautiful blue colour when in a layer several centimetres thick, but the colour is scarcely noticeable in a glass tube 1 mm. in diameter, and cannot be distinguished at all, even with the aid of a microscope, in a capillary tube 0·1 mm. broad. If the cell-sap of *Spirogyra* consisted of such a solution it would show no coloration, so that a colouring body may very easily penetrate the protoplasm without making itself apparent in the vacuole. PFEFFER, however, found that the roothairs of *Trianea* after a short time exhibited an obvious blue coloration when treated with a solution of methylene blue of 1 in 100,000, whilst in *Spirogyra* blue granular masses made their appearance in the cell-sap. Diffusion of the dye through the protoplasm must therefore not only have occurred, but a subsequent *accumulation* of the dye in the vacuole must also have taken place. This accumulation results from the transformation of the substance, after entry, into a form which cannot again pass out, thereby making room for the further entry of the pigment. This phenomenon is of the greatest significance in relation to the absorption of materials by the plant; for diffusion may result in the accumulation in the cell-sap of materials in request, until the degree of concentration inside is equal to that outside. Since, in most cases, the solutions available for the plant in nature are extremely dilute, only very small amounts of material can enter it by diffusion *only*, but if these substances so entering can be stored away in an insoluble, or, at all events, in another and non-diffusible form, continued entry of the material in question is possible.

The cell shows, therefore, certain characteristics which are of fundamental import in the well-being and life of the plant; one of these is its capacity for absorbing materials, not indiscriminately just as they are presented to it, but selectively, both from a qualitative and quantitative point of view. Hence a substance widely diffused in nature may be altogether wanting in the cell, simply because it is not capable of diosmosis, whilst a comparatively rare substance may be accumulated in considerable quantity. It is only in a few cases that we are exactly acquainted with the cause of the storage, e.g. that of methylene blue by the root-cells of *Lemna*. In this case the dye is united with tannin, and tannate of methylene blue is incapable of penetrating the protoplasm in any direction. No absorption or storage of this body occurs if the cell be placed in tannate of methylene blue instead of methylene blue itself, nor does any exosmosis of the tannate formed in the cell occur if the cell be again placed in water. If, however, a drop of citric acid be added to the water, after a short time the blue colour gradually disappears inasmuch as a process the converse of storage takes place. First of all a very little citric acid enters the cell and unites with the methylene blue; as a result of this union, room is created for the entry of more citric acid, and as citrate of methylene blue is capable of penetrating the protoplasm, all the blue colour in the cell in the long run undergoes exosmosis. Storage of such compounds does not always take place in such a simple manner as that just described; sometimes the changes are less, some-

times more extensive. As an example of an elaborate alteration we may take the formation of the insoluble, and therefore osmotically inactive, starch from sugar absorbed by the cell, while the precipitates by ammonium carbonate already alluded to, removable by mere washing in water, may be quoted as a simple illustration of the same phenomenon. In most cases we are unacquainted with the mode in which storage in the cell-sap is effected. For instance, when a nitrate or other inorganic salt accumulates in the cell-sap and reaches a higher concentration there than in the surrounding fluid, it is not impossible that a loose union occurs between that body and some other, but such unions are not very probable. When unilateral accumulation occurs without alteration of the substance, *purely physical diffusion conditions*, which we have hitherto accepted as essential, *cannot be operative*, or, at least, not entirely so. On this question we must await the results of further inquiry. NATHANSOHN (1902) has made an attempt in this direction, but his results cannot be said to be above criticism. He experimented with *Codium tomentosum*, the cell-sap of which he studied by quantitative chemical methods, but he overlooked the very large intercellular space system in that plant, which, in addition, communicates freely with the exterior, an omission which vitiates his researches. [NATHANSOHN has recently extended his osmotic studies (1904, Jahrb. f. wiss. Bot. 39, 607 and 40, 403). He finds that the permeability of protoplasm for any substance is not constant in degree but varies with external conditions. Protoplasm becomes, for example, impermeable to a substance if it be present in the vacuole in an amount bearing a certain quantitative ratio to the concentration of the same substance outside. This relation between the internal and external concentration may be again restored if the solution outside the vacuole be diluted, when a diffusion of the more dilute into the more concentrated solution takes place. In opposition to H. FISCHER (1904, Ber. d. bot. Gesell. 22, 485) who explains this phenomenon by the *Law of Distribution*, NATHANSOHN (1904, Ber. d. bot. Gesell. 22, 556) holds that it is due to variations in protoplasmic permeability, which cannot be accounted for in a purely physical manner.]

As a result of ingenious arguments, into which we cannot enter at present, PFEFFER has shown that the permeability of protoplasm does not depend on the protoplasm as a whole, but only on a very thin, microscopically indistinguishable layer, which may be termed the plasmatic membrane. An *outer* plasmatic membrane determines what substances shall enter the protoplasm, while an *inner* plasmatic membrane determines what shall enter the vacuole. These two membranes would appear to have different properties, since substances may enter the protoplasm in considerable quantities and yet bring about plasmolysis, owing to the fact that they are unable to penetrate the inner plasmatic membrane. Sugar, as we shall see later, behaves in this way in many cases.

Further, a *single* cell not infrequently contains several vacuoles whose contents differ from each other, and the plasmatic membranes of these vacuoles have in all probability different permeabilities. The cell's organization, as HOFMEISTER (1901) has shown, must operate so as to keep the varied chemical products apart, and here the plasmatic membranes must be of service; should these membranes alter in character, the previously separated products may come in contact with and react upon each other, and hence the variations in the plasmatic membranes must be of fundamental importance in the life of the cell.

Finally, we must inquire into the causes of varying permeability of protoplasm to different substances. So far as purely physical conditions determine permeability, a survey of diosmotic substances first of all may possibly give us a clue. OVERTON'S (1899) comprehensive observations, made with the aid of various methods, enable us to present the following summary :—

A. Members of the **aliphatic series** which *pass through* the protoplasm in so far as they are soluble in water :—

I. *Easily diosmotic* :—

1. Univalent alcohols (methyl alcohol, ethyl alcohol, allyl alcohol, ethyl ether).
2. Aldehydes (formaldehyde, chloralhydrate).
3. Ketones (acetone, sulphonal).
4. Halogen-hydrocarbons (chloroform).
5. Neutral esters of inorganic and organic acids, provided with one O–H group.

II. *Not readily diosmotic* :—bivalent alcohols (glycol) and the amides of univalent acids.

III. *Diosmotic with difficulty* :—trivalent (glycerine) and quadrivalent alcohols (erythrite), urea.

IV. *Scarcely diosmotic*:—hexivalent alcohols, hexoses, amido-acids, neutral salts of organic acids.

B. Among **substances which do not belong to the aliphatic series,** and which readily enter the protoplasm, the following are known :—

Benzol, xylol—anilin, formanilide, acetanilide—phenol, resorcin, orcin, phloroglucin—antipyrine—free alkaloids, but not their salts—basic aniline dyes, but not their sulphur containing salts.

The *ready solubility* of all these substances in *ether, fatty oils,* and similar media is, according to OVERTON, characteristic, and this authority has assumed that the limiting layer of the protoplasm is impregnated with a substance with a similar power of solution, and thus that only those bodies which are capable of solution in the limiting layer can enter the cell, that is to say, the osmotic peculiarities of the plasma depend on phenomena of *selective solubility* (compare TAMANN, 1892). OVERTON gives many examples of the similarity existing between solutions in oils and in the plasmatic layer ; he shows especially how by certain substitutions many bodies can be made to enter the plasma by dissolving them in oils.

Certain poisons also, such as corrosive sublimate, iodine, picric acid, and osmic acid which, owing to the rapidity with which they penetrate the protoplasm, have been considered as good fixing materials (compare p. 9), are shown by OVERTON to be readily soluble in oil. Most salts, however, are insoluble in oil, and appear to be incapable of penetrating the protoplasm. Further research on this subject is urgently needed, for it will appear later that many of these inorganic salts are indispensable and must be absorbed from without. Further, accurate research is still required on the mode of absorption of the ordinary gases of the atmosphere, viz. oxygen, nitrogen, and carbon-dioxide. That these gases do penetrate the protoplasm cannot be doubted, as our exposition of gaseous exchange in the plant will show.

It is, however, by no means probable that the plasmatic layer consists of a fatty oil, since Algae, for example, are capable of living for whole days without suffering injury in a 2 per cent. solution of sodium carbonate, a substance which would emulsify, and therefore destroy the oily layer. OVERTON therefore came to the conclusion that the plasmatic membrane derived its osmotic characters from the presence of a large percentage of cholesterin and lecithin, and this hypothesis he has elaborated in his more recent researches (1900), where he investigates the solubility of various substances in cholesterin. He finds that the solubility of various bodies, more especially aniline dyes, in cholesterin corresponds much more closely with the absorption of such substances by the protoplasm than with their solubility in oil. The presence of cholesterin in the plasmatic layer would thus explain the absorption of oils (Lecture XIII)

and xylol (OVERTON, 1899), and also of substances insoluble in water, while it would present no obstacle to the passage of water. It is worth noting in this connexion that lanolin, a derivative of cholesterin, is able to absorb more than double its weight of water.

Although OVERTON'S hypothesis appears in many respects inviting, still it has not been proved either in principle or in detail. In the first place, there are *specific* differences in permeability; *Penicillium*, for instance, will not permit the entry of copper salts, although these are readily absorbed by the majority of plants. Similarly *Beggiatoa* is able to take up sulphuretted hydrogen, although Algae closely allied do not do so. Such differences may be explained by assuming specific chemical constitutions for the plasmatic membranes of the species concerned. Moreover, the plasmatic membrane of one and the *same individual* varies according to external conditions. These variations may depend on changes taking place from time to time in the cholesterin-lecithin mixture, although it is possible that, under certain conditions, other substances may play a part in the constitution of the plasmatic membrane. Further, we cannot escape from the criticism that the plasmatic membrane may often be in our experiments not quite in a natural condition. For instance, many plasmolysing substances are instrumental in forming precipitation membranes on the surface of the plasma, and thus it is possible that we might be studying the characters, not of the plasmatic membrane in its natural state, but those of a precipitation membrane artificially produced (BERTHOLD, 1896, p. 152).

[WÄCHTER'S researches (1905, Jahrb. f. wiss. Bot. 41, 165) have shown that exosmosis of sugar from the cells of the onion is prevented by salt-solutions, but that quantities of sugar diffuse from cells when these are surrounded by water. In the case of beet also he has observed exosmosis of sugar to take place, in opposition to the results which DE VRIES obtained (p. 13). WÄCHTER'S work does not, however, provide us with an explanation of the complicated osmotic phenomena seen in beet, although his studies demonstrate very clearly that the osmotic peculiarities of the plasmatic layer are exceedingly variable (compare Lecture XIV).]

As a matter of fact, a renovation of the plasmatic membrane has been experimentally established. It occurs, for example, on the surface of the protoplasm which exudes from a wound in *Vaucheria*, and may be observed also after injuries inflicted on plasmodia of Myxomycetes. In the latter case it may be easily shown that new plasmatic membranes are formed on isolated parts of the general cell plasma (PFEFFER, 1890`

Bibliography to Lecture II.

BERTHOLD, E. 1886. Studien über Protoplasmamechanik. Leipzig.
HOFMEISTER, Fr. 1901. Die chemische Organisation der Zelle. Braunschweig.
NÄGELI. 1855. Pflanzenphys. Unters. 1, 21.
NATHANSOHN. 1902. Jahrb. f. wiss. Bot. 38, 241.
NERNST. 1890. Zeitschr. f. physik. Chemie, 6, 37.
OVERTON. 1895. Vierteljahrsschr. d. Naturf.-Gesell. Zürich.
OVERTON. 1899. Ibid.
OVERTON. 1900. Jahrb. f. wiss. Bot. 34, 669–701.
PFEFFER, W. 1877. Osmotische Untersuchungen. Leipzig.
PFEFFER, W. 1886. Unters. aus d. bot. Instit. Tübingen, 2, 179.
PFEFFER, W. 1890. Plasmahaut und Vakuolen (Abh. math.-phys. Kl. Sächs. Gesell. 16, 187).
TAMANN. 1892. Zeitschr. f. physik. Chemie, 10, 255.
DE VRIES, H. 1877. Die mechan. Ursachen d. Zellstreckung. Leipzig.
DE VRIES, H. 1884. Methode zur Analyse d. Turgorkraft. Jahrb. f. wiss. Bot. 14, 427.

De Vries, H. 1884 a. Bot. Ztg. 46, 229.
De Vries, H. 1888 b. Ibid. 46, 393.
De Vries, H. 1889. Ibid. 47, 309.

[A complete exposition of osmosis and related phenomena may be found in B. E. Livingston's treatise, The Role of Diffusion and Osmotic Pressure in Plants. Chicago. 1903. See also Höber, 1902, Physikalische Chemie der Zelle und der Gewebe. Leipzig.]

LECTURE III

THE ABSORPTION OF WATER

Leaving now the consideration of the simple relationships of the single cell which, when surrounded by water on all sides, is able to absorb water with dissolved gases and solids, dependent on the degree of permeability of the protoplasm, we have next to inquire into the mode of absorption of materials by the complex plant body. Examination of such a cell-complex as we meet with in the higher Algae (Florideae, Fucaceae) or in a submerged or floating Phanerogam (e. g. *Lemna triscula*), discloses a superficial cell layer whose individual units are, so far as absorption of materials from without is concerned, in all respects comparable to the solitary independent cells of which we have already spoken. Beneath the superficial cells, however, we find internal cells which are prevented from obtaining direct access to the external medium, and are dependent on such materials as have been permitted to pass through the cell-walls and protoplasm of the more peripheral cells. The *external cell layer*, in the first instance, determines what substances shall pass into the internal cells, although not all substances capable of entering the superficial layer necessarily pass farther inwards. The internal cells may in a sense be considered to be in direct relation with the external medium, since they are in connexion with it through their cell-walls, in which, generally speaking, all the substances in question—more especially water—can move.

In principle this is true of all land plants, and one might affirm that a cell in the topmost bud or leaf of an oak-tree was in direct communication, through the cell-walls, with the aqueous solution in the soil in which the roots are imbedded, although thousands, or even millions of cells intervene between it and the tips of the roots. In practice, however, this case is quite different from the preceding one, since further exposition will show that any exchange of materials is impossible by this means, owing to the enormous distance which separates the units. Hence we must regard the absorption of materials in the *land plant* as quite distinct in character from that occurring in a *single submerged cell*. No physiological researches are requisite to prove that the two regions of the terrestrial plant, manifestly different even to the non-botanist—viz. the root imbedded in the soil and the shoot expanded in the air, are essentially different in their methods of absorbing nutriment. The root absorbs the water present in the soil and the substances dissolved in it in the way already described, while the shoot absorbs the materials in the air in the gaseous form essentially. Naturally, therefore, in the following pages we must consider separately those constituents of the higher plant which are derived from the soil and those which are absorbed from the air.

First of all the plant absorbs *water* from the soil, which, as is well known, is indispensable to all organisms and to the vegetable kingdom in particular. Apart altogether from the fact that the chemical elements which go to form water, i. e. oxygen and hydrogen, form, in combination with carbon, the most important constructive units in organic compounds, *water itself* is an indispensable constituent of all cell membranes, which, as a matter of fact, are, in the

living state, invariably saturated with water of imbibition ; in the second place, the protoplasm of the living cell is also always saturated with water, and in the third place, the vacuoles, which frequently constitute the greater part of the cell, consist mainly of water. Again, chemical analysis (p. 5) has shown that parts which appear to contain little or no water do, as a matter of fact, contain it in considerable quantities. Were the plant to be as sparing in its use of water as it is of nitrogen (Lect. XI), absorption of water would be proportional to the addition of new members to the plant body ; but such economy is by no means the rule. On the contrary, the plant is, at least under certain conditions, lavish in its use of water. The large amount of water which the plant has extracted from the soil by means of the roots, with the expenditure of much energy, it sends back again into the air in the form of water vapour. According to HABERLANDT (1877), a plant of maize in the course of the summer gives off into the atmosphere 14 kg. ; a hemp plant gives off 27 kg. ; and a sunflower, 66 kg. ; i. e. in each case several times the weight of the plant. All these plants are small ; what then must be the amount given off by a tree ? The following data, which we owe to v. HÖHNEL'S very careful calculations, may be quoted in this relation. A large birch tree with, say, 200,000 leaves, gives off in the course of the season 7,000 kg., or about 38 kg. per day. A 110-year old beech tree gives off, in round numbers, 9,000 kg. in the course of the summer, and, on the basis of 400 such trees to the hectare, a wood of that size (two and a half acres) would give off 3,600,000 kg. of water in one season. Although these numbers make no claim to absolute accuracy, still they give us a rough idea of the very large amount of water concerned in this process.

Our first task must be to investigate the means by which the plant is able to absorb so vast an amount of water from the soil. With this subject is intimately connected the transpiration of water vapour from the leaves. Since the parts which are concerned in the absorption of water and those concerned with its transpiration are situated far apart, to obtain a complete picture of the whole process of water circulation in the land plant we have to consider as well the mode of *conduction* of water.

The soil from which the normal terrestrial plant obtains its entire water supply consists of a mixture of mineral detritus and the remains of organisms (humus). The individual constituent particles are of very variable size, loosely or compactly arranged, but in all cases so aggregated that interspaces occur between them, spaces which we will assume, to begin with, are full of air. If rain falls on such a soil, or water reaches it from somewhere else, the air is completely driven out of the interspaces between the soil particles, which then become filled with water. If the subsoil be impermeable to water, for example if it consists of clay, this condition of things becomes permanent and a marsh arises, characterized at once by the *abundance of water* and the *scarcity of air*. The abundance of water enables the plant to recoup itself with ease for the water it has lost, and hence it might be imagined that such a soil would form an ideal habitat for a plant. Experience teaches us that this is far from being the case. Certain plants only, and amongst cultivated plants only a few (e. g. rice) are able to thrive in marshy soils, or even tolerate them for any length of time, whilst the majority of our economic plants are killed in the presence of superabundance of water, and thrive only in soils with a moderate supply (WOLLNY, 1897). The reason for this is, not that there is too much water, but that certain accessory conditions are not fulfilled. That the injurious effects associated with marshy conditions are due to the poisonous influence of putrefying substances is a common belief, but no good evidence in support of this view is as yet forthcoming (WACKER, 1898). The only other possible reason for the injurious influence of a marsh on land plants is the absence of oxygen. As a matter of fact, water-culture experiments teach us

that there are very few land plants which are capable of developing normally when their root-systems are submerged in such water-culture solutions (Lect. VII), for under these conditions the only oxygen available is that dissolved in the water—an amount far below that present in a well-aerated soil. In water-cultures, moreover, the root is always able to find some free oxygen, whilst in swampy situations the minutest traces of this gas are often used up. Swamp plants overcome this difficulty by providing themselves with an elaborate intercellular space-system by means of which the gas may enter the root from above, and frequently by the formation of special respiratory roots which project above the medium (GOEBEL, 1886, 1887; JOST, 1887; KARSTEN, 1892).

Let us now consider the case where the subsoil is permeable to water. The water soaking into the superficial layers of the soil will partly, at least, drain into the deeper layers and carry the air with it into the larger interspaces, but all of it does not percolate downwards in this way. Some remains adherent to the individual soil particles, while more collects in the minute cracks and cavities in the particles and is firmly held there by capillarity. The amount of water which remains in the soil as compared with the volume of the soil is termed the soil's *water capacity*. Water capacity varies with the nature of the soil, and also, and chiefly, with the number and size of the spaces existing between the soil particles. This variation, within wide limits, is very considerable. The following data will serve to illustrate this fact :—

Water capacity of different soils (AD. MAYER, 1901).

	Vol. %
Humus soil	55
Clay soil	53
Fine sandy soil	30
Coarse ,,	10

Water capacity of quartz soils (WOLLNY in RAMANN, 1893, p. 67).

	Vol. %
Size of grain 1·00–2·00 mm.	3·66
,, ,, 0·25–0·50	4·38
,, ,, 0·11–0·17	6·03
,, ,, 0·01–0·07	35·50

These amounts of water are retained by soils only immediately after a thorough soaking; part is lost again in the process of evaporation. Plants, however, during their vegetative period, when most water is required, are frequently obliged to obtain it from a relatively dry soil, and hence they must be provided with a greatly branched root-system with the utmost possible absorbent surface.

The importance of the root for the purpose of water absorption is evidenced by the fact that in the seedling the root is driven into the soil long before the leaves unfold. In a word, water is what the seedling primarily needs, since all the other substances required by it are supplied in abundance by the reserves stored up in the cotyledons or in the endosperm. In many cases the primary root derived from the radicle of the seedling remains active for many years, or, it may be, for life; it grows in length and may pierce the soil to a very considerable depth, should the nature of the deeper layers permit. In desert plants especially, tap-roots of enormous length may be developed, which are primarily of service in the absorption of water from the deeper layers of the soil. As a general rule, however, the primary root is not the only active agent; it is aided by a series of lateral roots, which arise from it in acropetal succession, sometimes almost at right angles from the parent root and penetrating the soil horizontally, or curving downwards at angles of 70°, 60°, or 50°. In many plants, e. g. in *Vicia faba*, according to HELLRIEGEL (1883), the main root shows continued growth after the appearance of the lateral roots, whose degrees of development are approximately indicative of their age, those nearest the apex being the shortest, those farthest away the longest, whilst the apices of all of them lie approximately on the surface of an imaginary cone, whose apex is the tip of the main root itself. Another type of root-system is exemplified by the yellow lupin. Here the lateral roots are much fewer in number and more

irregular, in the first instance forcing their way to a moderate depth beneath the surface of the soil, while the older roots rapidly fall behind in rate of development. Again we meet with a third type where, while the earlier stages in development are similar to those already described, the tap-root later on surrenders its prominent position, then ceases to grow, and finally becomes altogether abortive. To this type of root-system, found in trees more especially, we will recur again presently. In the fourth type the primary root is wanting from a very early stage, and is replaced by a tuft of lateral roots, equivalent in value, which arise from the base of the stem. Examples of this type are furnished by grasses and bulbous plants. For descriptions of the root-systems of herbaceous plants, a subject on which very little research had hitherto been carried out, reference may be made to the comprehensive work of FREIDENFELT (1902).

The root-systems of trees deserve special mention for two reasons; first, because these plants go on growing for many years, and because the enormous amount of water used up by the crown of foliage imposes a specially heavy demand on the activity of the root. Thanks to the elaborate researches of NOBBE we are able to form a fairly good conception of the root-systems of the pine, the fir, and the spruce. NOBBE (1875) cultivated seedlings of these plants during one summer period in large glass vessels filled with sand, and, when autumn arrived, estimated the number of roots and their collective length after washing out the entire root-systems. Some of the data he obtained are summarized in the subjoined table :—

	Number of roots.			Total length of roots in mm.		
	Fir.	Spruce.	Pine.	Fir.	Spruce.	Pine.
1. Rank (chief root) . .	1	1	1	300·0	290·0	873·0
2. ,, . .	48	85	404	636·0	1333·5	4438·5
3. ,, . .	85	162	1955	56·0	312·5	5491·5
4. ,, . .	0	5	749	·0	5·0	1143·3
5. ,, . .	0	0	26	·0	·0	41·5
Total .	134	253	3135	992·0	1941·0	11987·8
Proportional value .	1	2	24	1	2	12

These three one-year-old plants, grown under exactly similar conditions, exhibit highly striking differences, both in the number of their branch roots and also in the total length of their root-systems. The sum of the lengths of all the roots is, in round numbers, in the fir, 1 m., in the spruce, 2 m., in the pine, 12 m. If estimated in surface measurement, we obtain areas of, in the fir, 49·52 sq. mm., in the spruce, 64·33 sq. mm., and in the pine, 142·23 sq. mm. Similarly, when the absorbing surface is considered, we find that the pine again stands far ahead of the fir and the spruce. The mass of soil entangled in the roots of the pine forms, according to NOBBE, a cone, 80–90 cm. in depth, and with a surface area of 2,000 sq. cm. If we divide this cone into layers, each 10 cm. in thickness, we find 1,548 lateral roots in the uppermost layer, and, successively downwards, 217, 446, 366, 121, and 38 lateral roots. The pine is thus in contact with a very considerable mass of soil by means of its root-system; thus it is able to put the soil to greater account, and so may succeed in growing in what is otherwise an unfavourable situation. Its alleged indifference to its surroundings is thus shown to be due to its great power of making the best of things. The behaviour of the plant in later years differs very considerably from that of the seedling. A vigorous outgrowth of lateral roots near the surface of the soil, in the case of the pine seedling, is suggestive of the subsequent feebler power of growth on the part of the main root and

the formation of a root-system distributed widely in an almost horizontal direction; the tap-root, however, still remains in existence. The tap-root of the spruce certainly penetrates the soil at first deeply, but after five years or so it ceases to grow, so that the tree in later stages of development is quite superficially rooted. The fir alone becomes a deeply-rooted tree with a predominant tap-root. An example may now be taken from deciduous trees. The beech, according to HARTIG (as cited by C. KRAUS, 1892), bears in its first year a simple tap-root with a few lateral branches. By the third year the most superficial of these take on vigorous growth and develop into a richly branched root-system close to the surface of the soil. By the fifth or sixth year the tap-root, which has now attained a length of at most ½ m., ceases to grow, and only the lateral roots go on developing. Up to an age of thirty years, two or, more rarely, three of the deeper seated lateral roots develop pre-eminently, pushing their way obliquely into the deeper layers of the soil. After that, onwards, the more superficial roots overtake them as far as rate of growth is concerned, and spread themselves out horizontally beneath the surface of the soil, constituting the chief part of the root-system. Hence when the tree is felled the root-system is found to form an unusually shallow layer in comparison with its horizontal extension, being at most 60 cm. deep.

Since the time of HALES (1748) many estimates have been made as to the extent of the root-system of different plants as well as with regard to the amount of soil laid under contribution by them. Thus NOBBE (1872) has shown that the aggregate length of all the roots of a one-year-old wheat plant amounts to 500–600 m.; that of a pumpkin may reach 25 km. (SACHS, 1882, p. 19), while SCHUMACHER (1867) has made measurements by *weight* of the root-system of several cultivated plants. SACHS (1882, p. 19) estimated the *space* occupied by the roots of a sunflower at one cubic metre, so that one may safely conclude that the root-system of a large tree is distributed through hundreds of cubic metres of soil. Inquiries of this kind, however, are, from the physiological point of view, not of much service since, as is well known, all roots have not the same functions. In perennial root-systems we may distinguish conducting and absorbing roots. The former are the permanent parts of the root-system, soon becoming covered with cork on their outer surfaces and taking no further part in the *absorption* of water; they serve to fix the plant firmly in the soil, however, and also to carry the absorbent roots. The latter are thin and remain so, and after a certain time disappear. These are the roots which absorb the water, although by no means their entire surface subserves this purpose—only that of the extreme apices where these are covered with hairs, or where hairs have not yet developed (KNY, 1898). Roothairs are always wanting in some land plants, and the general epidermis performs the function of water absorption in such cases. Apart from such exceptional conditions we may designate the roothairs as the special organs for the absorption of water. The roothairs are tubular prolongations of epidermal cells, sometimes of considerable length, which have the effect of increasing very considerably the absorbent surface of the root. F. SCHWARZ (1883) has reckoned that the surface of the root of the maize is increased five and a half times by the formation of roothairs, of barley, twelve times, and of *Scindapsus*, eighteen times. New roothairs are developed acropetally from day to day on the elongating root as the older hairs die off behind—for the roothairs live for only a short period. Regions covered by dead hairs absorb water only with difficulty, so that we must calculate the area of the apical regions as well as the increase of surface due to roothairs, if we are to arrive at a correct estimate of the region of functional activity of the root. Such estimates have not as yet been made.

If we now inquire how the individual roothair absorbs water from the soil it will aid us considerably if we study carefully SACHS'S statement on the subject (1865) taken in conjunction with an examination of Fig. 5. This

figure shows the surface cells (*ee*) of a root, from one of which a single root-hair (*hh*) has grown out. 'The bodies deeply shaded are microscopically minute particles of soil between which are shown airspaces left white. Each soil particle is surrounded by a thin layer of water held fast by surface attraction; where the attraction of neighbouring particles of earth co-operate at their re-entering angles these, otherwise thin, layers of water form thicker accumulations. These aqueous spheres are indicated in the drawing by wavy lines. The surface of the roothair is also (at *a*) covered by a thin layer of water, and its walls are saturated with it. Let us now regard the roothair for a moment as inactive, and assume that no disturbance at all is taking place in the soil; then all the aqueous spheres of the soil particles are not only in contact with each other, but are also in equilibrium.'

'If we now assume that the roothair, *hh*, absorbs water at *a*, its surface layer in that situation will have less water than corresponds to its power of attraction; it withdraws water first of all from its immediate neighbourhood and, in consequence, the equilibrium in these situations will be disturbed. This disturbance spreads outwards on all sides until the molecular equilibrium of all the aqueous spheres is re-established. By this means they all become thinner and thinner and the soil as a whole drier. This desiccation, however, may make itself evident not merely in the immediate neighbourhood of the roothair, but will at the same time affect more distant parts. Every roothair becomes thus a centre of a current directed towards it from all sides, and at the surface of a small root covered by thousands of roothairs a similar movement results which directs the aqueous particles in the soil from all sides, but more especially radially, towards the axis of the root.' The root is thus capable of making use of layers of soil although it may not actually be imbedded in them. 'If we assume the aqueous envelope of a particle of soil to consist of several very thin layers, then the aqueous molecules lying nearest to the particle of soil will be attracted with maximum force, and this attraction becomes progressively less in the successive external layers until, in the outermost layer, when the soil is saturated with water, the molecular attraction is only just great enough to prevent the water from trickling away. When the water disappears at *a* or at *β*, *γ*, &c., the outermost layer of the aqueous spheres, more especially, moves first, because it is the one least firmly held and most easily put in motion. The more water the roothair has already taken up the thinner are the aqueous spheres of the entire system, and the greater is the force with which the primary layer—now outside—is held; so much the greater must the force be which can pull the water into the wall of the roothair, and the more difficult and slower the transmission of a disturbance from *a* to *β*, *γ*, *δ*. A condition of the aqueous envelopes may finally ensue where all the primary layers are held so firmly by the soil particles that no more water can enter the wall of the roothair.'

Fig. 5. Roothair, *hh*, in the soil. Diagrammatic. For explanation see text (simplified from SACHS'S Experimental Physiology). In the figure the thickness of the adhesion layers is greatly exaggerated; they cannot be distinguished microscopically.

When this degree of drought in the soil is reached then the aerial parts of the plant must obviously wither though transpiration be prevented as much as possible. SACHS found withering took place in tobacco plants grown in different

kinds of soil, with different amounts of water, even when the leaves were exposed in the dark to a damp atmosphere. The following table shows this more in detail:—

Kind of soil.	Original amount of water in g. in 100 g. of dry weight.	Amount of water in g. present in soil when the plant withered.
Mixture of sand and humus .	46·0	12·3
Loam	52·1	8·0
Coarse quartz sand. . .	20·8	1·5

100 g. of soil contained in the first case 12·3 g., in the second 8 g., and in the third 1·5 g. of water, which were not available for the plant's use. These quantities correspond approximately to those left in the soil when it is air-dried.

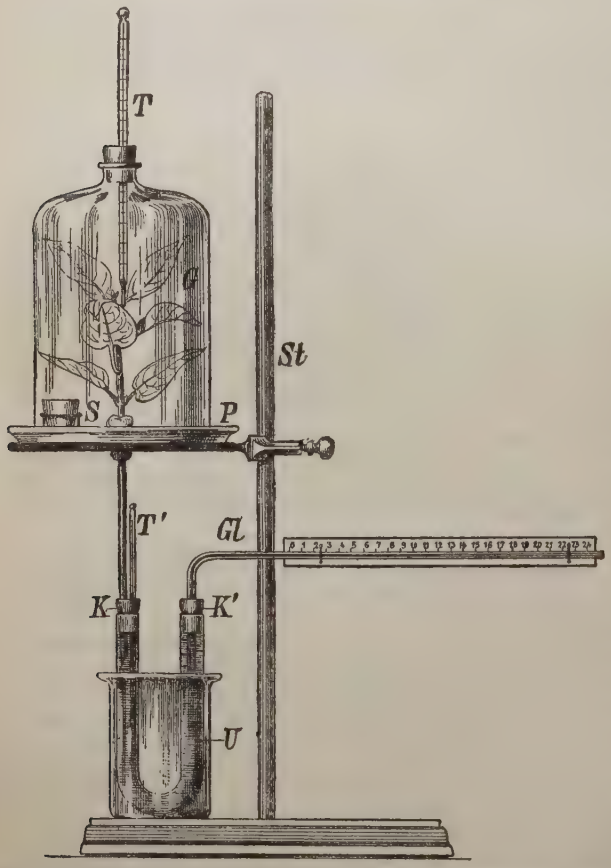


Fig. 6. Potometer. (From DETMER'S Smaller Practical Physiology, 1903.)

By what force then does a roothair overcome the adhesion of the water to the soil particles? After what we have learned as to the osmotic characters of a single cell, we can have no hesitation in ascribing this power to osmotic activity. In fact, the plasmolytic method enables us to demonstrate with ease an osmotic pressure in the roothairs. Owing to this osmotic pressure, as we have seen, the cell-wall will be stretched until its elastic expansion equals the turgor, and the water will be sucked into the cell cavity so enlarged by the stretching of the wall, as by a suction pump. The cell-sap will first of all withdraw the water from the protoplasm, which, in its turn, owing to its power of imbibition, will endeavour to draw fresh supplies of water from the membrane. The wall will then contain less water than it can hold in virtue of its power of swelling, and in consequence will suck up the water which we have spoken of as adhering to the soil particles.

If now the water osmotically absorbed by the roothair be retained, movement of water, after a time, on the restoration of equilibrium, must come to an end. In reality the movement of the water is not completely stopped, a condition of equilibrium only is reached in which the osmotic entry of water is balanced by the outflow effected by the pressure of the cell-wall. However,

owing to the fact that the aerial parts are transpiring, as well as to other causes, the epidermal cells of the root in ordinary land plants are always absorbing water, and hence a continuous inflow of water is kept up. If the supply is sufficient to replace loss by transpiration, then the amount of water in the plant is thus approximately a constant quantity. As the soil becomes drier the absorption of water becomes, as we have seen, increasingly difficult and the plant begins to wilt. The absorption of water by the root is influenced to a very considerable extent not only by the amount of water present in the soil but by other external factors as well. Thus it has been long known that low temperatures, from + 4° to + 2° C., cause certain plants, e. g. tobacco and pumpkin (SACHS, 1860), to wither, and even to die, if the exposure to such temperatures be prolonged. These low temperatures act injuriously on the plant, very often not directly, but by *retarding the absorption of water* (KIHLMANN, 1890). Strictly speaking, the withering is due not merely to a diminution in the *absorption*, but possibly also there may be an interference with *conduction* somewhere; at all events the direct influence of temperature on absorption itself still wants elucidation. KOSAROFF (1897), to whom we owe investigations on this subject, employed a simple apparatus, known as a potometer, which we shall find of service in other investigations later on. The principle of this apparatus is explained by Fig. 6. Into the end of the U-tube a branch is inserted through a cork, *K*, care being taken that the junctions are airtight; into the other end is inserted, also through a cork, *K'*, a capillary glass tube, *Gl*, bent at right angles and having a graduated scale attached to it. Each absorption of water by the plant manifests itself by a backward movement of the thread of water in front of the scale. The apparatus may be improved by placing the root-system of a growing plant into a large glass vessel, holding a water-culture solution, instead of into a U-tube (Lecture VII). By means of a funnel, suitably fixed, and provided with a stop cork, it is possible to replace periodically the water lost, so that the experiment may be carried on for a longer time.

When the root-system of *Phaseolus multiflorus* was placed in this apparatus and kept at 20·8° C., KOSAROFF observed that in twenty minutes the meniscus in the capillary tube had moved through 210 mm. At 0° it moved only about 140 mm., and other experiments gave quite similar results. The amount of water absorbed at 0° C. was only three-quarters to two-thirds of that absorbed at 20° C.

How is this to be explained? If we assume transpiration interrupted, a certain time will have to intervene before the cells of the root reach osmotic equilibrium, that is to say, until they have absorbed as much water as corresponds to their osmotic activity. The *amount* finally absorbed by the cells when equilibrium is fully re-established will be practically the same whether the temperature be 0° C. or 20° C., but the *time* which elapses before the re-establishment of equilibrium will depend very materially on the temperature. As a matter of fact, the osmotic pressure alters according to temperature in the same way as gas pressure; but as this alteration does not amount to more than $\frac{1}{273}$ per degree we need not consider it any further, since it is of no physiological importance. RYSSELBERGHE (1901), by observing plasmolysis and recovery, has estimated the time taken by water in passing through the protoplasm in different cases, and has arrived at the following results :—

Temperature	0°	6°	12°	16°	20°	25°	30°
Rate of movement of water	1	2	4·5	6	7	7·5	8

At 30° C. the movement of water was eight times as rapid as at 0° C. At first sight this result appears remarkable, and does not agree with the behaviour of PFEFFER'S osmotic cell; for the copper-ferrocyanide membrane exhibits no such irregularities in behaviour at different temperatures. RYSSELBERGHE, however, referred the matter to purely physical causes, and he remarks

that, according to differences in temperature, gelatine also shows variations which are very noticeable, but always less so than those exhibited by protoplasm in its resistance to the passage of water. Nevertheless it appears to us that the part played by the protoplasm in the absorption of water is not purely physical. It is certainly remarkable that the rate of water transference increases only slowly between 20° and 30°, and it is unfortunate that RYSSELBERGHE did not employ still higher temperatures in his researches, seeing that, as we shall have frequent occasion to note in the course of these lectures, the vital activity of the plant increases in intensity proportionally as the temperature increases, until a degree is reached somewhere between 30° and 45°, when it again decreases. Certain observations of KOSAROFF pertinent to this question tend to support this view. Withering soon takes place if a stream of carbon-dioxide or hydrogen be passed through soil in which healthy pot plants are growing; absorption of water is therefore retarded by this means as well as by low temperatures. This result may be observed within an hour after the initiation of the experiment, and the carbon-dioxide itself could scarcely have caused death in that time. The hydrogen is not so rapid in its action; we know for certain that it is itself harmless, but operates only by displacing the oxygen. It would appear, therefore, from the evidence afforded by these researches, that the absorption of water is retarded by suppressing the supply of oxygen to the root. Oxygen, as we shall see later, is an indispensable factor in a large number of vital processes, although of no account in the diffusion of water through a dead membrane; we are thus compelled to believe that living protoplasm plays a great, but as yet unknown, part in the absorption of water. The fact established by KOSAROFF that dead roots take up less water does not necessarily prove this, for death itself will doubtless induce purely physical changes in the plasmatic layer.

Biologically it is of the greatest interest to note that all plants are not injuriously affected to the same degree by low temperatures; many, indeed, can still absorb water from a frozen soil (KOSAROFF, 1897).

The root is the normal organ for absorption of water in ordinary land plants, hence plants whose roots have been destroyed invariably die from want of water, even though their shoots be frequently watered by rain or dew. We must not, however, conclude from that, that aerial organs are altogether *unable* to absorb water. The epidermal cells of the leaf, just as much as those of the root, contain osmotically active substances in their vacuoles, and must also be able to absorb water if only the outer wall be permeable to it, and if sufficiently large quantities of water derived from rain or dew can accumulate on the leaf. Not infrequently, however, the shape and arrangement of leaves (STAHL, 1893) are adapted to the rapid drainage of surplus water from the leaf surface. Thus, according to STAHL'S researches (1897), the position of leaves on the axis is such as to prevent or hinder the formation of dew upon them (Lect. XXXIX); finally anatomical adaptations occur, especially coatings of wax, which render the leaves waterproof. Such peculiarities in structure are of restricted occurrence, but aerial parts of plants differ in general from subterranean parts in possessing a cuticle formed from the outermost layer of the cell-wall. This cuticle is not only of maximum thickness on the stems and leaves, but exhibits varied physical and, probably also, chemical peculiarities. The cuticle of the shoot only has hitherto been studied in sufficient detail, that of the root is still urgently in need of investigation. A research of this kind has been carried out by KRÖMER (1903, Bibl. botan., Heft, 59) in the Marburg Institute, and he holds that the root epidermis has an outer wall of very varied composition and structure, but that a *genuine* cuticle, such as occurs in the epidermis of the stem and leaf, is entirely *absent*.

The cuticle has been found to consist of a material which shows a strong resemblance to cork, agreeing with that substance in possessing the physical

characteristic of being capable of swelling very slightly in water, and hence of permitting very little water to pass through it. The cuticle of the root, on the other hand, is easily permeable to water and swells up into a gelatinous mass (SCHWARZ, 1883). Even the almost waterproof and strongly cutinized cell-walls of the leaf of *Sedum fabaria* do not appear to be quite impermeable to water, since WIESNER (1882) has observed experimentally that the leaves of this plant, if immersed in water, increase in weight. By means of an old experiment (HALES, 1748, p. 78), easily repeated without special appliances, direct absorption of water by the leaf may be demonstrated. If part of an amputated leafy twig be immersed in water, leaving the cut end and some of the leaves exposed to air, the latter go on transpiring but remain turgid all day long despite the fact that water is being given off from the exposed regions—so demonstrating that the submerged leaves are able to absorb as much water as is given off by the exposed leaves. The success of the experiment in any given plant depends entirely on the relative numbers of the absorbing and transpiring leaves. WIESNER (1882) dipped only the apices (bearing some young leaves) of amputated twigs of the vine in water, leaving most of the older leaves to transpire into the air. The result was surprising. The exposed leaves remained turgescent, whilst the apical leaves, although submerged, wilted. In this instance the amount of water absorbed by the apical leaves was insufficient to cover the loss sustained by transpiration. The older leaves withdrew water from the cells of the apex of the branch, and caused them to wilt, even though in water all the time. From what has been said it would appear that aerial parts of plants are also capable of absorbing water, and it would not be difficult to bring forward evidence from the literature on the subject to show that not only leaves and young stems, but also bud-scales and older branches, whose cuticle has been replaced by the still more impermeable cork, can absorb water (KNY, 1895). In our ordinary terrestrial plants, however, during the rainy period, the amount of water absorbed is quite insufficient to cover the loss due to transpiration, and hence the absorption of water through the shoot may, for all practical purposes, be ignored. In tropical regions, with a much greater precipitation of moisture, with frequent downpours of rain and greater general dampness of the atmosphere, innumerable plants exist which do not come into contact with the soil at all, and hence can obtain their water supplies from the air only. There are, for instance, the epiphytes, living on the tops of trees, whose biological peculiarities have been described for us in a most attractive manner by SCHIMPER (1888) and GOEBEL (1889). While making a general reference to the works of these authors, we must confine ourselves here to mentioning only a few examples. In the case of many of these epiphytes, as for example, the Araceae and Orchidaceae, long aerial roots are formed whose function it is to absorb water from the air. These roots differ widely in structure from ordinary subterranean roots. Instead of a single layered epidermis producing roothairs, we find a many-layered cellular envelope, the units of which have lost their protoplasm at an early period of life, and which now form air chambers, communicating with each other and with the exterior by means of pores. When rain falls on this sheath, the drops sink into it as into a sponge, replacing the air in the otherwise empty cells ; from thence the water readily penetrates to the living cells of the root cortex beyond.

In other epiphytes the roots are reduced in size and serve merely as holdfasts, whilst absorption of water is carried out by the leaves alone. This is seen best, for example, in many Bromeliaceae, where the leaves are often arranged in a rosette, their bases enclosing a funnel-shaped cavity in which rain-water accumulates as in a cistern. Hairs of special character, quite distinct from roothairs, absorb the water in the pitcher. [These hairs have been lately studied by MEY (1904, Jahrb. f. wiss. Bot. 40, 157) and STEINBRINCK (1905, Flora, 94, 464).]

SCHIMPER has proved that the amount of water absorbed from the funnel exactly corresponds with that lost in these plants from transpiration, whilst their roots are quite unable to provide water in quantity sufficient for their needs. On the other hand, there are some forms provided with *special* holdfasts and which have lost their *roots* altogether. The best known of these rootless Bromeliaceae is *Tillandsia usneoides*, whose long, grey, tail-like masses occur in tropical and sub-tropical America in such quantities that they actually obscure the foliage of the trees on which they are epiphytic. 'The first beginning of a tuft is, as a rule, the separation of a solitary twig which twists round another branch of the tree; from it arise numerous lateral twigs, some of which become themselves propagative shoots, although most of them develop quite freely into the air.' The leaves of this *Tillandsia* form no collecting funnels, they are certainly not arranged in a rosette, but come off *individually* from the stem and are small and inconspicuous. The *whole plant* is covered with water-absorbing hairs such as occur on the leaf bases of other forms, and these give it its grey colour. In general appearance, as indeed its specific name, 'usneoides,' indicates, it resembles an indigenous lichen, also a pendent epiphyte from trees. This recalls to us the fact that epiphytes occur in our own climate also, although these are almost entirely confined to plants of low grade, viz. mosses and lichens. The feature in which these plants have an advantage over higher forms and which qualifies them to withstand our dry seasons, is not any specially economical management of the water absorbed, but a capacity for being able to withstand *desiccation*, a capacity, however, by no means confined to epiphytic forms. These plants may often become so dry that they may be actually crumbled into dust, and that, too, without losing their vitality. As soon as the first drops of rain fall on them and they have absorbed as much as they require, they start life afresh. Perhaps the best examples of this peculiar mode of life are to be found among the crustaceous lichens which grow over the walls of old houses or on bare rock. Such forms often obtain in a few hours or days only, during the course of months, all the water they need for carrying on their vital functions, and in the interval are completely dried up by the sun's heat. Not only is this capacity for resisting desiccation of the very greatest importance to these plants, but the ability they also possess of absorbing the first traces of water after long drought is of deep significance. Their cell-walls in the dry condition remain capable of being easily wetted, and rapidly take up water once more, differing in this respect from the dust of our streets, which in losing water loses also its capacity for quickly reabsorbing it. Owing to this characteristic, mosses and lichens play a very important part in the economy of nature, inasmuch as they are able to store up rain, forming living water reservoirs whose contents are for a long time of benefit to other organisms. Without going into further details we may, in conclusion, merely allude to the fact that other epiphytes which are unable to withstand desiccation, are at first entirely absent from regions subject to periodic deficiency of water. Further they are compelled to exercise great economy with the store of water which they have collected during the rainy period, i. e. they must limit their transpiration greatly or provide themselves with special water reservoirs. Many varieties of such reservoirs have been described by SCHIMPER and GOEBEL, as well as by other earlier writers.

Bibliography to Lecture III.

FREIDENFELT. 1902. Flora, 91, 115.
GOEBEL. 1886. Ber. d. bot. Gesell. 4, 249. 1887. Bot. Ztg. 45, 717.
GOEBEL. 1889. Biologische Schilderungen. Marburg.
HABERLANDT, F. 1877. Wiss.-prakt. Unters. auf d. Gebiete. d. Pflanzenbaues, 2, 158.
HALES. 1748. Statik der Gewächse. Halle.

HELLRIEGEL. 1883. Beitr. z. d. naturw. Grundlagen des Ackerbaues. Braunschweig.
HÖHNEL. 1879. Wollny's Forsch. auf d. Geb. d. Agrikulturphysik, 2, 398.
JOST. 1887. Bot. Ztg. 45, 601.
KARSTEN. 1892. Bibliotheca botanica, Heft 22.
KIHLMANN. 1890. Pflanzenbiol. Studien aus Russisch Lappland.
KNY. 1895. Ber. d. bot. Gesell. 13, 361.
KNY. 1898. Ibid. 16, 216.
KOSAROFF. 1897. Einfl. äuss. Faktoren auf d. Wasseraufnahme. Diss., Leipzig.
KRAUS, C. 1892. Wollny's Forsch. auf. d. Geb. d. Agrikulturphysik, 15.
MAYER, Ad. 1901. Agrikulturchemie, 5th ed., II, 1, 154.
NOBBE. 1872. Landw. Versuchsstationen, 15, 391.
NOBBE. 1875. Tharandter forstl. Jahrb. 201.
RAMANN, E. 1893. Forstl. Bodenkunde u. Standortslehre. Berlin.
RYSSELBERGHE. 1901. Bull. Acad. Belg. (Sciences), 1901, Nr. 3. (Recueil Inst. bot. d. Brux. 5, 209.)
SACHS. 1860. Bot. Ztg. 18, 123.
SACHS. 1865. Handb. d. Exp.-Physiol. (Hofmeister, Handb. d. phys. Bot. 4). Leipzig.
SACHS. 1882. Vorlesungen über Pflanzenphysiologie. Leipzig.
SCHIMPER. 1888. Die epiphytische Vegetation Amerikas. Jena.
SCHUMACHER. 1867. Jahresb. f. Agrik.-Chemie, 83.
SCHWARZ, F. 1883. Unters. aus d. bot. Inst. Tübingen, 1, 135.
STAHL. 1893. Annales Jard. Buitenzorg, 11, 98.
STAHL. 1897. Bot. Ztg. 55, 71.
WACKER. 1898. Jahrb. f. wiss. Bot. 32, 71.
WIESNER. 1882. Sitzungsber. Wiener Akad. 86, 40.
WOLLNY. 1897. Forsch. auf d. Geb. d. Agrikulturphysik, 20, 52.

LECTURE IV

TRANSPIRATION

AFTER this brief reference to epiphytes we may now return to the consideration of ordinary land plants, of which our trees and cultivated plants may be taken as examples. The soil, we have seen, supplies them with the water they require, and that they absorb by the root; we must now study the reverse process, viz. the giving off of water by *transpiration* from parts above ground, more especially from the leaves.

No special methods are required to demonstrate this phenomenon, for just as a free water surface, a sponge saturated with water or damp soil, gives off water vapour into the air, provided the latter be not itself saturated, so too must also the plant, containing as it normally does abundance of water. And just as under natural conditions the water evaporated is not always replaced at once, so, too, transpiring plants exhibit great variations in the amount of water they contain, variations often so obvious as to be noticeable to the naked eye. Who has not noted herbaceous plants and even trees with limp leaves or flowers on a hot day in July? The wilting is simply the indication of the suppression of osmotic distention of the cell-walls and, consequently, of tissue tensions, owing to the loss of water. So long as the loss of water keeps within certain limits, a renewed supply of water can once more induce normal turgescence; consequently, we notice that not infrequently during the night, when transpiration is reduced by lowering of the temperature, the leaves again become rigid. Not only from such everyday experiences, but also from the fact that we can prevent wilting by placing the plant in the shade, or by watering it at the right time, even the 'man in the street' can appreciate the importance of external conditions in determining the amount of transpiration. Before passing to the consideration of the question as to how far the plants themselves and how far external factors influence transpiration, we may glance

at the ways in which the existence of this phenomenon may be proved, and the more exact methods employed to estimate minute losses of water, not merely the grosser evidence presented by the process of withering.

Thanks to the large number of experimental researches that have been carried out on the subject from the days of HALES (1748) up to the most recent times (e. g. BURGENSTEIN, 1887–1901), we have become acquainted with so many methods that we must limit ourselves in their enumeration. Evaporation from a plant may be demonstrated in the clearest and simplest way by observing the dampness deposited on a bell jar placed over it and kept at a low temperature. The reason for this deposition of moisture is the same as that for the dimming of a window pane when one breathes on it ; it is nothing more or less than the deposition of dew on a cold surface. The most exact, uniform, and quantitative method of proving the existence of transpiration is to employ a balance. If proper precautions be taken to permit of water being given off from the plant only, and not at the same time from the earth in which it is rooted, it may be shown that the decrease in weight from hour to hour is due to loss of water. It is true that there are other processes taking place in the plant which lead to change in weight, still, quantitatively speaking, they are insignificant when compared with the change in weight due to movements of water. The data given on p. 25 as to the amount of transpiration have been obtained by weighing. A third method, extremely convenient and useful for demonstration, consists in making use of the alteration in colour which many substances undergo when they absorb water. STAHL (1894), to whom we owe the application of this excellent method of investigation, used strips of filter paper soaked in cobalt-chloride. ' Cobalt paper ' is deep blue when dry, but becomes red when wet. The method of use is to place a small piece of the blue paper on the subject of investigation—say a leaf—covering it up with a glass plate so as to eliminate the influence of atmospheric moisture. According as the leaf gives off much or little water the paper changes in colour, after a few seconds, hours, or days. In place of change of colour we may employ bodies which exhibit hygroscopic movements, such as gelatine (BENECKE, 1899) or awns of *Erodium* (DARWIN, 1898), for the demonstration of the same phenomenon.

Many authors, e. g. VESQUE (1877), MOLL (1884), BONNIER and MANGIN (1884), and KOHL (1886), have employed the potometer figured on p. 30 for demonstrating transpiration. It will be remembered that with this apparatus it was the amount of water *absorbed*—not the amount *given off*—that was measured. If transpiration be kept within moderate bounds, however, one can make out that the two amounts are equal—that the loss due to evaporation is covered by the amount absorbed. The potometer method has many advantages ; it is very easily demonstrable, more so if coloured water be employed in the capillary tube ; it is very convenient, especially if the influence of external factors on transpiration have to be studied ; it does not necessitate the presence of roots on the plant—isolated branches are quite sufficient for the purpose.

With the aid of one of these methods let us, first of all, study the effect which the *plant's structure* has on transpiration. Observation alone teaches us that the external walls of the epidermal cells are the parts of the plant first concerned in the giving off of water vapour. Like all other cell-walls these contain water of imbibition, and this water is retained with a certain amount of force. Every particle of water lost by evaporation is replaced by another which the wall attracts from the protoplasm. The protoplasm in turn abstracts water from the cell-sap. But the cell-sap also holds the water firmly, and, in consequence of its osmotic properties, together with the imbibitive energy of the cell-wall and protoplasm, the outer surface of the plant gives off less water vapour than an aqueous surface of equal extent under the same conditions. AUBERT (1892) found that, taking the evaporation from a water surface, per unit of

time, as 1,000, the evaporation from an equal surface of a dilute solution of gum was 843, of malic acid 837, of glucose 773. An *Opuntia*, on the other hand, gave off only ten units of water per unit of time. A special factor must, however, be taken into consideration, viz. the cuticle, already described in speaking of the absorption of water, whose influence is in the direction of retarding transpiration. Since the cuticle imbibes little or no water, it acts like a film of oil spread over an aqueous surface. The differences between varieties of cuticle have been already referred to, and these are of the greatest importance in relation both to the absorption and giving off of water. The thin and gelatinous external walls of the root and of submerged plants are very permeable to water, so that these parts readily dry up and wither when exposed to air, and between this condition and the other extreme, where the cuticle is thick and practically impervious to moisture, as in hard leathery leaves, every possible transition occurs. Some numerical idea of the action of the cuticle may be obtained from a study of some of BOUSSINGAULT'S results (1878). He experimented on apples which were in part provided with a normal cuticle and in part had the cuticle removed. A square centimetre of normal apple surface lost 0·005 g. of water per hour, whilst the skinned apple lost 0·277 g., or fifty-five times as much.

Such investigations, however, take for granted that the cuticle over the exposed part is a continuous layer and destitute of all apertures, but this is by no means true of all cuticles. In very many cases the cuticle is pierced by microscopically minute but extremely numerous holes; the otherwise continuous epidermis is interrupted by special organs, the *stomata*. Each stoma (Fig. 7) consists of two cells (guard-cells) which differ from other epidermal cells in their curved form. Owing to the fact that these cells have their concave sides turned toward each other, a small slit is left between them, opening on the one side to the air and on the other into a large intercellular space, known as the 'respiratory cavity' (see Fig. 7, *B*), standing in direct communication with the general intercellular space-system in the body of the plant. The spaces found between the cells in the plant's interior are not, however, completely shut off from each other, but form an intercommunicating system of chambers and canals, constituting the *aeriferous system* of the plant. By means of the stomata this system is put in direct communication with the atmosphere.

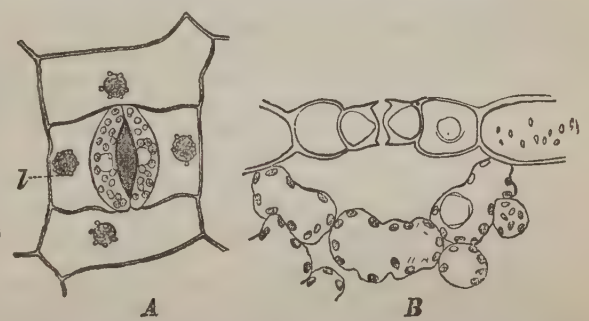


Fig. 7. Lower epidermis of *Tradescantia virginica*. *A*, from above; two guard-cells centrally placed: *B*, in section, showing below the guard-cells the respiratory cavity, on which the chlorophyll-bearing parenchyma abuts. × 240. (From the Bonn Textbook.)

The stomata, the exits of the aeriferous system, permit gases of all kinds to enter the plant as well as to pass out, and direct gaseous exchange may, by this means, take place between cells deep in the interior of the plant and the air. We can easily convince ourselves of the value of the stomata and intercellular space-system for such exchange by placing a leaf from an appropriate plant in water and arranging that the atmospheric pressure on the end of the submerged petiole is less than that on the leaf blade. As a result of a quite insignificant difference of pressure—mouth suction is often sufficient for the purpose—a continuous stream of air-bubbles may be seen escaping from the petiole. It can be at once demonstrated that this air has entered by the

stomata, for if these be blocked up by vaseline or tallow the stream of bubbles at once ceases. It must also be noted that other exits from the aeriferous system, in addition to stomata, are known to occur in plants. ('Pneumachodes'; compare HABERLANDT, 1896).

At the present moment the only gaseous exchange taking place through the stomata which we need consider is the giving off of water vapour. It follows from the structure of the plant as described above that, in addition to the transpiration taking place from *epidermal cells*, there must also be a certain amount of 'internal transpiration', since each cell wherever it borders on an intercellular space will give off water vapour, the immediate result being the saturation of the air in the intercellular spaces. The whole plant thus loses water whenever water vapour escapes through the stomata from the intercellular spaces. Obviously only a minute quantity of water can pass to the exterior through a single stoma since the slit is so small. The diameter of the largest of them (e. g. those of *Amaryllis*) is only 0·01–0·02 mm., and the openings are so minute that a needle prick appears as a huge hole in comparison. The significance of the stomata in the vital economy of the plant, apart from characteristics which will be studied later, depends on their *immense number*. In the situations in which they are most abundant, e. g. the underside of the foliage leaf, we find on an average from 40 to 300 per sq. mm., but in extreme cases there may be as many as 625 (*Olea*) or 716 (*Brassica rapa*) in the same area. According to NOLL (1902) an average-sized leaf of *Brassica rapa* has no fewer than eleven millions of stomata, while a leaf of the sunflower has thirteen millions. It must be remembered in this connexion that BROWN and ESCOMBE (1900) have shown that diffusion of gases through a plate pierced by numerous fine pores takes place as rapidly as if the spaces between the pores were non-existent.

We must also differentiate between an epidermal and an intercellular, or, in other words, between a *cuticular* and *stomatal transpiration*, and the difference may often be recognized by contrasting the behaviour of opposite sides of the leaf. Many foliage leaves bear stomata only on their undersides, and if the cuticle be alike on both sides, we may assume that we have a cuticular transpiration taking place from the upper side, and a stomatal transpiration taking place from the lower *as well*. Several methods of investigation, most conveniently perhaps the cobalt-chloride method, demonstrate, however, that cuticular transpiration is frequently so small that it may be taken as practically nil. If a piece of blue cobalt-chloride paper be placed on the underside of a leaf of *Liriodendron tulipifera* it becomes red in a few seconds, while a piece placed on the upper side remains blue for several hours, the general conditions being the same in each case. Plants which live in damp air, e. g. the Hymenophyllaceae, possess much thinner cuticles than those which live in drier air, hence in their case cuticular transpiration is very obvious and readily capable of demonstration by cobalt paper. The extreme case is shown by submerged plants and by roots where the permeability of the cuticle (in the absence of stomata) to water is shown at once by the rapidity with which wilting takes place.

Even assuming that the nature of the cuticle, and the number and dimensions of the stomata in any plant are known, the absolute amount of transpiration cannot be determined without knowing as well what the *external conditions* are, since the amount of transpiration varies extremely with alterations in these. The way in which many of these external factors affect transpiration is obvious, for they may be observed just as readily in purely physical experiments with a substance capable of absorbing water, e.g. glue or filter paper; in the plant, on the other hand, the influence of external factors introduces remarkable complications, due to the fact that the organization of the plant is, in the first instance,

altered by them, and this in turn affects the amount of transpiration. Among physical influences the *dampness of the air* holds a foremost place; its effects are so obvious that further explanation is unnecessary. Similarly with *temperature*; every increase in temperature must cause an increase in transpiration —every decrease must retard it. If the plant be at a higher temperature than the environment it can still give off water vapour into the air even though the latter be saturated; this higher temperature is attained by respiration or by the absorption of light and heat rays, often aided by the presence especially of colouring matters (STAHL, 1896). Transpiration is also increased by *oscillation*, for the plant is thus taken out of the saturated atmosphere produced over its surface in consequence of transpiration, and brought into a new region not yet saturated. The same effect is produced by moving the air rather than the plant, and hence every breath of *wind* aids transpiration. The *dampness of the soil* has an influence which is somewhat less obvious. Dry soil hinders transpiration because it retards absorption of water; owing to the lack of a reserve of water the cell-sap becomes more concentrated in the transpiring organs, and hence is less ready to give off water vapour into the air. Concentrated salt-solutions, if the root has to absorb water from them, act in the same way as a dry soil, although *dilute* solutions also have an influence on transpiration which has not as yet been fully explained. Dilute acids retard, and dilute alkalis accelerate, transpiration. Probably in these cases the explanation is not purely physical, but must be sought for in alterations in the characters of the plant itself, having their cause especially in the activities of the guard-cells of the stomata.

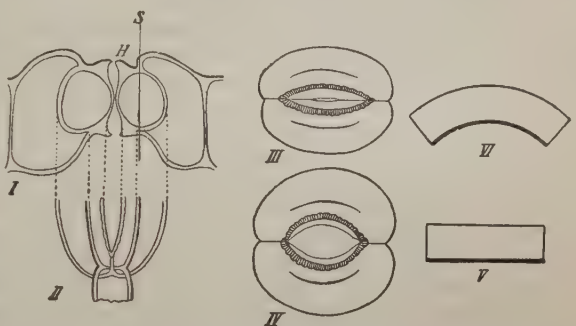


Fig. 8. *I–IV*, stomata of *Amaryllis formosissima* (after SCHWENDENER). *I*, in section; *II*, surface view of half-stoma; *III*, surface view of a closed, and *IV*, of an open stoma; *V*, *VI*, for explanation see text.

We have not as yet discussed this question of the activity of the guard cells, and so far, our treatment of the subject would suggest that the stomatal aperture was always of the same size. That is, however, by no means the case. On the contrary, the guard-cells are capable of opening and closing the stomatal slit according to conditions, and thus of allowing of the most varying amounts of transpiration, from nothing upwards. Variations in the size of the pore are attained by a very simple method, viz. by varying the degree of curvature of the guard-cells. To understand the mechanism of the process it will be necessary for us to study the structure of the stoma somewhat more in detail than we have already done. We may select for detailed study the stoma of *Amaryllis*, whose structure has been elucidated most thoroughly by SCHWENDENER (1881). Other plants exhibit other adaptations than those seen in *Amaryllis*, but the mechanical *principles* involved are *fundamentally* the same for all (compare HABERLANDT, 1896, and COPELAND, 1902). Fig. 8 shows a stoma of *Amaryllis*, both in the open and in the closed condition, in surface view and in transverse section. The latter (Fig. 8, *I*) shows the asymmetrical form of the guard-cells in relation to the line *S*, which separates the concave from the convex side. While the convex half forms almost exactly a half circle, the outer contour of the concave side is much more complicated, and as a consequence the intercellular space between the concave edges of the guard-cells also

presents special peculiarities. On the outside, the lumen is constricted by horn-like ridges (*H*); then follows an enlargement known as the vestibule of the stoma. The vestibule again narrows to form the slit proper, followed by a further widening inwardly—the rear vestibule—once more contracted by a second pair of ridges. The inner contour does not, however, run parallel with the outer one, but forms almost a half circle. The concave wall is thus not of the same thickness throughout; it is relatively thin in the middle and thicker above and below where the ridges are (see Fig. 8, *I*). The occurrence of abundant chlorophyll in the guard-cells must be specially noted, the ordinary epidermal cells being as a rule destitute of green pigment. Further, the protoplasm of the guard cell encloses a large vacuole which is the seat of great osmotic activity. Under the influence of osmotic pressure the cell-walls are stretched, but the concave sides, owing to their greater thickness, are more able to resist this extension than are the convex sides, so that the stretching is more obvious on the latter than the former. The effect of this differential stretching is best illustrated by a model. If one takes a caoutchouc tube, completely closed, and having a strengthening layer pasted along one side, and forces into it air or water, though straight at first, it becomes bent (Fig. 8, *V*, *VI*). Now imagine two such tubes with the strengthened sides facing each other, united by their ends, but with the central region free; if turgor be produced in these tubes they will be seen to separate from each other in the middle. Much the same sort of thing takes place in the guard-cells of the stoma; increase of pressure induces the slit to open, as much as is shown in comparing Fig. 8, *III* and *IV*. We speak of the stoma in the first case as closed, in the second as open; as a matter of fact, however, when turgor is at a minimum, the two guard-cells do not lie so closely together as to hermetically seal the opening, although it is sufficiently obliterated to make the amount of water vapour which passes through scarcely worth speaking about; in other words, stomatal transpiration is almost as good as suppressed. By altering the pressure in the guard cells the plant is able to vary the width of the slit very considerably, and in this way the stomata become *regulators of transpiration* to a degree which would scarcely be anticipated.

The width of the slit does not depend entirely on the osmotic pressure in the guard-cells, but is also influenced by pressure in the cells surrounding them. If this pressure be eliminated, e.g. by pricking these cells, it will be seen that the stoma opens at once, although the pressure in the guard cells is not at the same time increased. Conversely also, increase in turgescence of the surrounding epidermal cells may bring about a passive closure of the stoma. Authorities differ in their estimates of the degree to which this activity in the general epidermal cells is functional *in nature*. SCHWENDENER (1881) held that it was of no importance, LEITGEB (1886) believed that it played a great part in the process, while DARWIN (1898) takes an intermediate position. So far as we are concerned we may take it that the active movements of the guard-cells alone are quite sufficient to account for the opening and closing of the slit. The effect of variations in pressure within the guard-cells may be readily demonstrated under the microscope. If the preparation shows the stomata open, addition of a plasmolytic solution will quickly cause them to close, and they may be made to open again at once by replacing the plasmolytic solution with water. In nature, variations in pressure so extreme as to lead to complete abolition of turgor, such as is effected by plasmolysis, do not occur; on the contrary a pressure of several atmospheres is maintained even when the stoma is quite closed.

The conditions under which opening and closing of the stomata take place are very different in different plants, still, *in general*, we may say that stomata in appropriate ways regulate the amount of transpiration, and that the plant is thus able to protect itself from wilting. Conditions connected with this

regulation of transpiration may undoubtedly occur which are dangerous to the plant, for we must not forget that the stomata are not merely the organs of transpiration, but are primarily the means by which carbon-dioxide enters the plant. Everything that retards transpiration must at the same time hinder the absorption of carbon-dioxide, and hence clashings may occur between the two processes, when the plant must naturally suffer from one cause or the other. Conflicting conditions of this kind are to be met with whenever we investigate natural phenomena closely.

We have now to study separately the more important factors which play a part in the movements of the stomata, and foremost among these we may place the *vapour tension of the atmosphere*. For purely physical reasons, abundance of moisture in the air must, as has already been shown, retard transpiration; but at the same time it will induce the stomata to open widely, and thus the physical condition will be in a sense neutralized. In dry air on the other hand, evaporation is increased, while transpiration is reduced because many plants close their stomata at once when wilting begins. It is easily seen how the degree of humidity of the air will act on the guard-cells sooner than on other cells; in damp air they attain the utmost osmotic turgor which the cell-sap is able to exert when water absorption is at a maximum; when loss of water takes place owing to increasing dryness of the atmosphere, if at the same time an abundant supply of water is not forthcoming, a rapid reduction in the turgor ensues, accompanied by closing of the stoma. Closure of the stoma is, however, by no means always a concomitant of wilting; in many plants the wilted leaf has its stomata wide open. Such leaves continue to give off water, and shrivelling of the leaf takes place with great rapidity. Plants of this kind can exist only in regions where the moisture is excessive, as, for example, among our native plants, those inhabiting marshes (*Alisma*, *Acorus*, *Menyanthes*, &c.) and shady places (*Osmunda regalis*). The cobalt-paper method will be found to be convenient in investigations of this sort, for by this method we may convince ourselves, for example, of a vigorous transpiration from the half-dried leaf in the plants above mentioned, where the stomata remain open, while in *Tropaeolum majus* this cannot be observed owing to the rapid closure of the stomata on wilting. Further, actual wetting will induce wider opening of the stomata than a damp atmosphere. This explains the result obtained by Wiesner (1882), who found that there was a marked increase in transpiration after the leaves had been dipped in water. Under certain conditions a quite contrary effect may be produced, as, for instance, when the neighbouring cells absorb water and the stomata are in consequence passively compressed (Kohl, 1886), or when the pores are blocked by capillary water.

The stomata of different plants do not always react in the *same* way to the second factor, which we may allude to here—namely, *light*. Frequently, e.g. in *Amaryllis*, *Aspidistra*, &c., we may observe that the slits open when the leaves are more brightly illuminated. Here again, since for purely physical reasons light furthers evaporation, there is the danger of excessive transpiration. In many cases withering of the leaves may be prevented in spite of the brilliant illumination, if the stomata close at the first indication of wilting. Stahl (1894) has proved that this result does not always follow, however, and it may be shown that if the leaf of *Tropaeolum*, slightly wilted, be exposed with closed stomata to direct sunlight, it does not wither any further even after some hours, although a fresh leaf exposed under the same conditions dries up rapidly, just because it does not close its stomata. We may conclude, therefore, that the action of light on the guard-cells is dependent for its effect on the chlorophyll contained in these cells. In fact, as we shall see later, the chlorophyll is able under the influence of sunlight to generate osmotically active substances, and hence, in a sense, to bring about opening of the stomata. Assuredly, however,

light operates still more in an indirect way, i. e. as a *stimulus* to the guard-cells. In many plants closing of the stomata has been observed to take place on darkening. *Darkening* must, therefore, also be recognized as a stimulus, since after osmotically active bodies have been manufactured in light, they cannot be removed so quickly, after exclusion of light, as to account for the rapidity with which the stomata again close. According to LEITGEB (1886) the closing of the stomata in the dark is passive, owing to the guard-cells being pressed together by the turgor in the neighbouring cells. An increase in turgidity in darkness is a very general and easily explicable phenomenon.

In addition to the effect of light and atmospheric moisture on the width of the stomatal aperture that of *changes of temperature* also has received attention, but into that and related subjects we need not enter, those already discussed being the most important. In spite of the voluminous literature on stomata, detailed researches on these organs in representatives of the chief biological groups of plants are still wanting. We know enough, however, to be able to affirm without fear of contradiction that in stomata plants possess a remarkably valuable apparatus for regulating transpiration, provided the external conditions for absorption and transpiration be approximately normal, that is to say, such as do not approach the extremes to which the plant cannot adjust itself. Any attempt to cultivate some of our economic plants, such as cereals or tobacco, under the conditions prevalent in a desert, or in the saturated atmosphere of a tropical forest would, undoubtedly prove a failure. At the same time, a study of plants which are naturally fitted to live under such extreme conditions discloses to us a variety of adaptations calculated, on the one hand, to limit transpiration as much as possible, and, on the other, to further it to the utmost. A brief sketch of these adaptations may be appropriately introduced here; the reader is referred for details to the works of HABERLANDT (1896), SCHIMPER (1898), and STAHL (1893, 1896).

Reduction in the amount of transpiration may be effected by a reduction in the number of stomata or by an alteration in their anatomical structure. Further, plants which live in dry regions usually have well-developed cuticles, whose power of retarding transpiration is increased by the deposition in, or on, them of wax; moreover, the capacity for imbibing water may be reduced in such parts of the plant as are exposed directly to air. Development of hairs full of air can also effectively retard transpiration, since such a covering protects the plant from the effects of air currents, producing a superficial region free from atmospheric movement. In addition to such preventatives dependent on cell structure we find also adaptations dependent on the form and arrangement of the parts concerned. A flat extension of the foliage leaf greatly favours transpiration, more especially if the whole leaf surface be exposed to the sun's rays; but plants are known to exist which avoid strong insolation by presenting the edges of their leaves to the sun (compass plants, *Eucalyptus*, &c., Lecture XXXVI), and especially, as need arises, by changing the position of their leaves with relation to the direction of the incident ray (Lecture XXXVI). More effective still is the power which certain plants possess of reducing their surface by rolling up leaves which are normally extended, or the extended form may be avoided altogether and a spherical shape assumed (Cactaceae, Euphorbiaceae, &c.).

On the other hand, in plants which inhabit very damp situations we find adaptations for furthering transpiration; as, for example, the special form and arrangement of leaves for aiding in the rapid drainage of water from their apices; coloured cell-sap and consequent increased temperature of the cells concerned; thin, easily permeable cuticle; increase of the epidermal surface; complete exposure of the stomatal guard-cells, &c.

It is of the utmost importance that the plant should be able, according to

variations in external conditions, to modify those structural adaptations which aim at aiding or retarding transpiration. This capacity the plant possesses during its development only, it is true, within certain definite limits (Lecture XXX).

From what has been said it will be seen that the amount of transpiration must vary greatly in different plants, and in the same plant under different conditions. Further, transpiration in individual organs varies very greatly. [Under ordinary circumstances transpiration from the *foliage leaves* is so pre-eminently greater than from other parts that we may really regard them as *the organs of transpiration*, and in consideration of the extent of surface they exhibit and the numbers of stomata they bear, this function is at once obvious.] Transpiration in the individual organ varies also with its developmental condition. Into these problems we need not enter here, but confine ourselves rather to inquiring whether such variations are bound up with the nature of the plant or whether they are accidental, in other words, whether the enormous exhalation of water vapour seen in certain plants is useful and essential to their welfare, seeing that others, especially submerged plants, can do without transpiration altogether. The answers given to this question have been by no means unanimous ; indeed some have been diametrically opposed to each other—one author holding that transpiration is a necessary evil (Volkens, 1887), another that it is vitally indispensable. Our experiences in these latter days have taught us, however, that there is nothing more likely to lead to error in the realms of physiology than the making of generalizations, since in more than one aspect differences in the most fundamental vital conditions have been shown to exist amongst organisms which outwardly give no hint of them. Without anticipating a detailed exposition later on, reference may be made here to certain lower plants, the condition of whose existence is a medium free from oxygen and which stand out in sharpest contrast to ordinary organisms which require free oxygen. It would certainly be quite wrong for us to conclude that, because individual plants can get on without transpiration, therefore transpiration was not essential to any. One thing is clear ; the entire structure of the land plant necessitates transpiration, since, were it to cease, the absorption and excretion of other gases would be impossible, and the life of the plant would come to an end. Plants inhabiting dry climates show us how far such a limitation of gaseous exchange can go. If we fail to find such protective adaptations against excessive transpiration in the majority of plants it must not be concluded that it would be impossible for these plants to develop such adaptations ; on the contrary, we must hold that they have no use for them. Finally, if we meet with plants which manifestly possess adaptations for accelerating transpiration, we ought to conclude that transpiration is a fundamentally important process in such plants. As a matter of fact, this view may be substantiated in several ways. There can be no doubt that transpiration markedly aids in the absorption from the soil, in large quantities, of the salts whose significance we shall have to discuss in an early lecture. [The influence of transpiration on the absorption of salts in the soil is very clearly demonstrated by Treub's researches on the absorption of potassium nitrate (1905, Annales Jardin bot. Buitenzorg, 2nd ser. 4, 119).] These salts are presented to the roots in very dilute solution, and it would take a very long time to transfer them to the highest branches of a tree by diffusion only. In reality these salt solutions are transported by way of the vascular bundles, and are carried right up to the cells of the leaf. Here evaporation takes place and a consequent concentration of sap and accumulation of salts. Moreover, there is another effect of transpiration which must not be overlooked. The leaves are exposed to sunlight and, since they are able to absorb light rays by means of their chlorophyll and, in certain cases, by means of other colouring matters also, they must become sensibly warmer. *Obser-*

vations show that the temperature of the plant is nevertheless on the whole about that of the air, and that can be possible only if cooling takes place after the increase in temperature due to the absorption of light rays. Heat, however, is necessary for evaporation of water, and hence transpiration must be accompanied by reduction of temperature. It should be remembered in this relation that we make use of this cooling effect of evaporation in everyday life when we sprinkle water about during a hot summer day, or when we store water in an unglazed earthenware vessel. If evaporation be thus a regulator of temperature in the plant, a considerable rise in temperature must be observable in feebly transpiring plants when exposed to the sun. ASKENASY (1876) was able to observe very high temperatures in certain oily plants. [URSPRUNG'S researches (1903, Bibliotheca botanica, Heft 60) may be compared with those of ASKENASY, which on the whole they confirm.]

	Temperature of plant.	Temperature of air.
Sempervivum alpinum	49·3° C.	31·0° C.
,, *sp.*	51·2° C.	
Aubretia deltoides	35·0° C.	
Sempervivum alpinum	52·0° C.	28·1° C.
,, *arenarium*	49·0° C.	
Opuntia raffinesquiana	43·0° C.	
Gentiana cruciata	35·0° C.	

While plants like *Aubretia* and *Gentiana* reach a temperature very little above that of the air, oily plants attain a temperature of over 50° C. This observation is of special interest when taken in conjunction with the fact that by far the majority of plants cannot endure so high a temperature. We see also how the power of resisting high temperatures must be a characteristic of such plants as are capable of living in dry climates, and that by no means all plants can adapt themselves to such vital conditions.

Bibliography to Lecture IV.

AUBERT. 1892. Annales d. sc. nat. VII, 16.
ASKENASY. 1875. Bot. Ztg. 33, 441.
BENECKE. 1899. Bot. Ztg. 57, Abt. II, 130, Note.
BONNIER and MANGIN. 1884. Annales d. sc. nat. VI, 17, 288.
BOUSSINGAULT. 1878. Agronomie, 6, 349.
BROWN and ESCOMBE. 1900. Phil. Trans. R. Soc. B. 193, 223.
BURGERSTEIN. 1887–1901. Materialien zu einer Monographie d. Transpiration, I, II, III, Verh. d. zool.-bot. Gesell. Wien, 47, 691 ; 49, 399 ; 51, 49.
COPELAND. 1902. Annals of Bot. 16, 327.
DARWIN. 1898. Phil. Trans. R. Soc. B. 190, 531.
HABERLANDT. 1896. Physiol. Pflanzenanatomie, sect. IX. 2nd ed. Leipzig.
HALES. 1748. Statik der Gewächse. Halle.
KOHL. 1886. Die Transpiration d. Pflanzen, etc. Braunschweig.
LEITGEB. 1896. Mitt. a. d. bot. Institut Graz, 1, 123.
MOLL. 1884. Archives néerlandaises, 18.
NOLL. 1902. Bonner Lehrbuch d. Botanik, 5th ed. p. 157. Jena.
SCHIMPER. 1898. Pflanzengeographie auf biolog. Grundlage. Jena.
SCHWENDENER. 1881. Monatsber. Berl. Akad. p. 833 ; Ges. Abh. 1898, 1, 33.
STAHL. 1893. Ann. Jardin Buitenzorg, 11, 98.
STAHL. 1894. Bot. Ztg. 52, I. Abt., 117.
STAHL. 1896. Ann. Jardin Buitenzorg, 13, 137.
VESQUE. 1878. Annales d. sc. nat. VI, 6, 183.
VOLKENS. 1887. Flora d. ägypt.-arab. Wüste, p. 51. Berlin.
WIESNER. 1882. Sitzungsber. Wiener Akad. 86.

[A very complete discussion of all the questions relating to transpiration will be found in A. BURGENSTEIN'S (1904) Die Transpiration der Pflanzen ; eine physiologische Monographie. Jena.]

LECTURE V

THE CONDUCTION OF WATER. I

SINCE certain parts of the plant *give off* water and other parts *absorb* it, *conduction* of water must of necessity take place in the regions lying between. Absorption, evaporation, and conduction of water may be carried out, under certain circumstances, in different parts of a single cell. On clay soils there is occasionally to be found an alga, *Botrydium granulatum*, which consists of a spherical green body, about the size of a pin-head, attached to the substratum by a number of colourless branches (Fig. 9). The spherical green region may be compared to the shoot of a higher plant, and the branched colourless region to the root, but the whole structure, being destitute of partition walls, may be looked upon in a sense as a single cell. Similarly the unicellular fungus, *Pilobolus*, has a root-system distributed throughout the substratum, and a club-shaped aerial portion at the termination of which the fruit is ultimately formed. Let us now suppose that transpiration is set up in one of those cells, which we will assume to be saturated with water; obviously the molecules of water will in the first instance be withdrawn from the *membrane* covering the aerial region; the membrane, in other words, loses its water of imbibition. In consequence of this, forces will be generated in the membrane which will induce a withdrawal of the water in the neighbourhood held firmly by the protoplasm. The protoplasm in its turn will seek to make good its loss by absorbing water from the vacuole, thus bringing about a higher concentration in the contents of the upper parts of the vacuole. Owing to diffusion, however, uniformity in concentration in the contents of both ends of the cell is re-established and the disturbance in equilibrium is transferred to the regions where renewed absorption of water from the soil may take place.

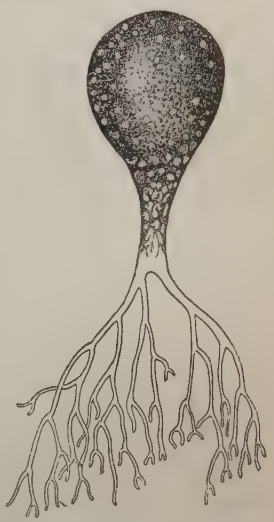


Fig. 9. *Botrydium granulatum*. × 25. (After ROSTAFINSKI in SACHS'S 'Lectures', first ed., fig. 2.)

Let us now consider a somewhat more complicated case by assuming that the *Botrydium* has been subdivided into two parts by a partition wall separating a green transpiring region from a colourless absorbing region. The first effect of transpiration will, just as above, lead to the concentration of the cell-sap in the green cell. This cell, however, abuts, not directly on water but on the colourless cell; from it, in obedience to the law of osmosis, the green cell will absorb water, and will continue to do so until the fluids in both cells attain the same degree of concentration. Equilibrium, however, is never reached so long as the green cell continues to transpire and so long as the colourless cell is in contact with water; for every withdrawal of water from the colourless cell is followed by absorption of water from the soil, and every entrance of water into the green cell is succeeded by renewed transpiration. In this illustration the osmotic attraction arising from the evaporation from one cell is simply transferred to the other, and thus there is really no essential difference between the first example we studied and this one. The case becomes slightly more complicated, if we take into consideration transpiration as exhibited by a multicellular fungus, such as *Penicillium*, a plant which is partly rooted in the substratum and partly exposed to the air.

Fig. 10 represents a small specimen of *Penicillium* with its horizontally placed hyphae imbedded in the substratum, and its erect conidiophores projecting into the air. For our present purpose we may imagine the organism simplified so as to be represented by the model illustrated at Fig. 11, where the cells of the filament, marked *A*, *B*, *C*, lie in the substratum, whilst the others, *a*, *b*, *c*, &c., are aerial. If now the cell *a* loses water in consequence of transpiration, it will at once endeavour to obtain a fresh supply from cell *b*. But *b* is also transpiring and will, in turn, abstract water from *c*. That another cell higher up than *b* is also endeavouring to obtain water is equivalent to imagining that *b* is transpiring more vigorously ; in other words, to the abstracting power of *b* exerted on *c* we must add the abstracting power of *a* also, and so on until we reach the cells which absorb the water from the medium. In these cells the converse process takes place to that exhibited by the transpiring cells. The sucking forces of all the cells, *a*, *b*, *c*, &c., are acting on *A*, while *A* replaces the water which it, in consequence, loses by abstracting water not only directly from the medium but also from the cell *B*. In this way the suck-

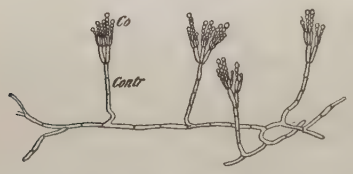


Fig. 10. Small plant of *Penicillium*. (After BREFELD.) *Contr.*, conidiophores; *Co.*, conidia.

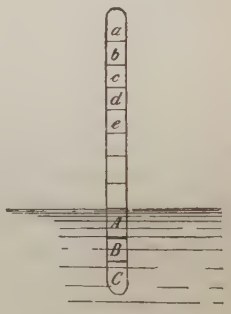


Fig. 11.

ing action of transpiration is transmitted backwards to all the cells which perform the function of roots.

As yet we have spoken only of those water currents in the organism which arise from the disturbance of osmotic equilibrium, and which continue to exist so long as there are differences in degree of concentration between the individual cells. If a large number of cells become united to form a simple or a branched cell thread or cell mass, we might conclude that any loss of water in consequence of transpiration from the superficial cells would be made good by water absorbed from the cells lying below. Looked at by itself this assumption would be correct if only the rapidity of the water currents due to osmotic activity were sufficiently great. Even although the *pits* found in the cell-walls aid in the passage of water, this is true only under special conditions and in relatively small cell-systems such as, for example, in *Penicillium*. Indeed the mode of *occurrence* of moulds in nature affords us most valuable suggestions on this subject, for we know that these organisms are confined to situations possessing a certain degree of atmospheric humidity ; we find that they prefer to live in small enclosed areas with stagnant atmospheres, and are quite unable to thrive where there is abundant aeration, even although all the other conditions necessary for their well-being are fulfilled. In these plants the absorption of water by osmosis takes place so slowly, that if the evaporation of water be increased even very slightly the transpiring cells are unable to obtain from below sufficient supplies to compensate them for what they have lost, and the plants in consequence perish from drought.

Under these circumstances one can very well understand that movement

of water from cell to cell in the higher plant cannot possibly suffice to replace the loss due to transpiration, and *experiments* have confirmed this conclusion. WESTERMAIER (1884) arranged strips of somewhat flaccid parenchyma taken from the water-bearing tissue of *Peperomia* and *Tradescantia* in such a way that one side only was in contact with water, and observed to what distance from the surface of the water the cells regained their turgescence. Although the nature of the external conditions made transpiration almost impossible, the height to which the water ascended never amounted to more than a few centimetres. Even the cells which were more than from about two to four centimetres distant from the surface of the water were unable, by means of osmotic suction, to attain their normal water capacity. From experiments of a similar nature, REINKE (1902) concluded that parenchymatous tissues of submerged plants, if one side be immersed in water, become dried up about two millimetres above the water level. Such experiments as these, coupled with observations on small multicellular plants, force us to the conclusion that in larger plants, trees especially, where the height of ascent of water is to be measured not by centimetres but by metres, the movement of water cannot possibly be effected by its transference from one living cell to another, but that conducting organs with special structure and activity must be forthcoming by means of which transport of water in quantity may be carried out.

These organs are the *vessels*. That this is the case may in the first place be concluded on purely anatomical grounds. The contents of the vessels consist, at least in part, of water ; their elongated form and the continuity of their lumina for long distances point emphatically to the conduction of water as their function. Not less suggestive is their distribution. They begin to appear in the centre of each root about the same distance from the apex as the roothairs do on the exterior ; and these roothairs are, as we have already seen, the water-absorbing organs. They stretch from this point upwards, uniting in their progress with side conduits from every lateral root, increasing in number and in total transverse section as they progress from apex to base. On reaching the stem they pass outwards into every branch, every twig, and every petiole. In all these organs the vessels are collected together into a few strands, but in the foliage leaves, the organs of transpiration, we find these cords resolving themselves into stouter or weaker subdivisions which are distributed throughout the leaf-blade in the form of a complicated network, so that every transpiring cell is in contact with a vessel either directly, or indirectly through the medium of a few parenchymatous cells. We thus arrive at the important conclusion, already recognized as fundamental on other grounds, viz. that transport of water from cell to cell is extremely limited, if indeed the cell bordering on a vessel can abstract water from it.

Physiological evidence as to the function of vessels may be most readily found in trees, since, owing to their often great height heavy demands are made on the vessels in such plants. Further, it is not unusually the case that both on the main trunk and on the branches there are regions of considerable extent which possess no lateral organs of transpiration and which are prevented from transpiring by their corky coverings. In such situations transport of water only, and no evaporation, can take place ; moreover, by breaking the continuity of the tissues in succession, we can determine with certainty which anatomical system is especially concerned in water conduction. Comparative examination shows us that the pith has nothing to do with water transport, seeing that in many cases it is absent altogether or consists of dried-up cells filled with air, or of parenchyma, with whose feeble power to act as a transporter of water we are already acquainted. It is quite otherwise with the cortex and phloem ; there is no want of elongated elements here running continuously for long distances, e. g. collenchymatous and sclerenchymatous tissue

and sieve tubes. That these elements have nothing to do with the transport of water in the stem is shown by a simple experiment, first carried out by HALES (1748) and repeated on numberless occasions since his time and always with the same result. In order to break the continuity two circular incisions are made round the stem right into the wood, and the intervening ring of tissue is removed. If this 'ringing' be not carried out too extensively, and if due care be taken that the stem does not become dried up or rotten at the region of ringing, the leafy crown will remain fresh for a long time, and the transport of water will not be interrupted to any appreciable extent by the ringing. We may conclude, therefore, that the conduction of the water is effected by the wood. In the long run, of course, it is impossible to prevent injuries to the exposed wood, when its capacity for conducting water will decrease and the leaf-bearing region above the ring dies after a few years, unless it has contrived meanwhile to make itself independent of the original root-system by the formation of a special new root-system above the excised ring. Observations made by TRÉCUL (1855) have shown how long a tree can, in spite of such treatment, remain alive above the point of ringing. A lime tree at Fontainbleau, for example, showed signs of life in its apex forty years after ringing had been performed.

From an examination of amputated branches, which, as every one knows, remain for a long time fresh, and are therefore capable of transporting water, one is easily able to conclude, by the method of exclusion, that the carriage of water takes place only by means of the woody tissue. If at the lower end a branch be cut in such a way that only the cortex, the pith, or the wood comes in contact with water, it will be found that in the first two cases the twig rapidly withers, but that it retains its vitality for a long period if the wood only be immersed. Examination of such branches further enables us to determine which part of the woody tissue is more especially concerned in water transport. Although *a priori* it would appear unlikely that the wood fibres or parenchyma should be the tissues specially concerned in the transport of water, still *direct* evidence that the *vessels* are the real agents is not at once attainable. The fact that water does ascend by the vessels, and more especially by their lumina, may be demonstrated (though the mode of proof is scarcely scientifically exact) by placing cuttings in a solution of an appropriate colouring matter (e. g. eosin) and permitting transpiration to take place. From the coloration of the walls of the vascular elements we may deduce the rapid ascent of the solution in the vessels, and all the more readily and clearly if we employ transparent white petals, the vascular network in which appears deeply stained by the pigment after transpiration has gone on for only a short time. Such investigations cannot, however, be carried out on the entire plant owing to the fact that the roots refuse to absorb colouring matters. Further, certain precautions have to be taken in experimenting with cuttings, of which we shall speak later. Obviously, then, the fact that the living wood elements are unable to absorb colouring matters leaves a doubt in our minds as to the validity of such experiments; for although under these conditions a movement of the colour solution is on the whole possible only in vessels which are destitute of protoplasm, and although its entrance proves more especially that it *can* ascend in the vessels, still these facts do not prove that in the *uninjured plant* it utilizes the lumina of the vessels *exclusively* for its ascent. Experiments where the lumina are occluded by the infiltration of foreign substances and thus made impassable for water are, for this reason, much more convincing. Thus ELFVING (1882) was the first to place cuttings in cacao-butter, which has a low melting point, and allowed them to transpire whilst standing in that medium, so that the butter rose in the cavities of the vessels. ERRERA (1886) employed melted gelatine for the same purpose. The infiltrated substances were thereafter coagulated by cooling, and complete occlusion of the lumina of the vessels was

thus obtained. Plants treated in this way, though once more placed in water, wilted with great rapidity; they were no longer capable of absorbing water, and the conducting power of the stem was destroyed. At the low temperatures at which gelatine and cacao-butter can be used, injury to the living cells is out of the question, so that it may be considered as certain that the vessels are essential to the transport of water and that that transport takes place by the *lumen* of the vessel, not as SACHS (1879) believed, by its *wall*. We must draw attention to the fact, however, that the walls of the vessels as well as the neighbouring parenchyma cells may also be concerned in the process; for our experiment only teaches us that the *lumina* of the vessels are essential, but gives us no information as to the participation of other elements in the conduction. By far the most convincing experiment is that which was made, first by VESQUE (1883), and later by KOHL (1886) and STRASBURGER (1891). It is possible, by means of a screw clamp, to compress the lumina of the vessels and so occlude them, the parenchyma being at the same time almost entirely crushed. So long as the vessels are squeezed in this way, the stem is unable to conduct water, but its capacity for doing so returns at once when, by removal of the pressure, the vessels regain their original shape by elastic recoil and once more exhibit distinct lumina. This experiment is most effective if water-cultures be employed, or if it be carried out on cuttings, with the aid of a potometer. Constriction of the lumina of the vessels at once makes itself felt, for the absorption of water rapidly sinks to nil; on the other hand, relaxation of pressure is immediately followed by the inrush of water into the vessels with increased rapidity. This tightening and slackening of the screw-clamp may be repeated many times, but always with the same result.

Before we make any attempt to examine more closely the ascent of water, we must inquire how the water succeeds in entering the vessels in the first instance. In the familiar experiments with amputated branches the water enters the cut ends of the vessels, very much in the same way as it enters a capillary glass tube whose open end has been submerged. In the normal plant, however, the vessels are both at their lower ends, and laterally as well, completely enclosed by living cellular tissue, and abut upon other vessels higher up, so that any water that succeeds in entering them must first of all have penetrated the living tissue by which they are surrounded. Now we have already seen in Lecture III how the cells of the root epidermis absorb water osmotically from the soil, and at the commencement of the present lecture we have determined under what circumstances water travels from cell to cell. The water absorbed by the epidermis is transferred to the centre of the root since the cell-sap is in a state of greater concentration there than it is in the epidermis, and it will continue to be so transferred until a similar osmotic pressure prevails throughout all the cells of the transverse section. Water in the same way will pass osmotically into segments of young vessels while these are still in an embryonic state and possessed of normal cell contents. When, however, a segment fuses with the next older segment an immediate dilution of its osmotically active cell-sap must take place since it is essentially *water* that is found in adult vessels. The question then comes to be how can water be abstracted from the cell-sap of a parenchymatous cell and transferred to the lumen of a vessel; one would expect the precisely converse process to take place. Before we attempt to answer this question it will be advisable to make ourselves thoroughly acquainted with the details of the process.

It is by no means difficult to demonstrate that water is given up to the vessels by the parenchyma cells. In many cases all that need be done is to cut off the aerial shoots of a herbaceous plant, when at once, or after a short time, a quantity of sap may be seen escaping from the wound. Owing to the turgidity of surrounding parenchyma, sap may also be squeezed out of the

cut ends of latex tubes, sieve tubes, and similar elongated elements, which, in consequence, lose their turgescence. This particular instance, however, is not apposite in this relation. Similarly the contents of the vessels may be squeezed out by the pressure of surrounding parenchyma, more especially if the vessels be young; at a later stage the secondary thickenings in their walls renders this impossible. Nevertheless it is obvious that only a small amount of sap can pass out in this way. The following are the amounts of water which HOFMEISTER (1862) found were ejected from the root-systems of *Urtica urens* and *Solanum nigrum*:—

Plant.	Time in hours.	Sap given off in cmm.	Volume of root in cmm.
Urtica urens . .	99	3025	1350
,, ,, . .	40	11260	1450
Solanum nigrum .	48	1800	1530
,, ,, .	65	4275	1900

These observations are specially valuable in consequence of the fact that the volume of the roots from which the water was excreted was noted as well. The table shows that in a few days the root gives off several times its own volume of water. During that time it must have been absorbing new supplies of water from the soil, and it can, after even a longer period of activity, still maintain its original amount of liquid contents.

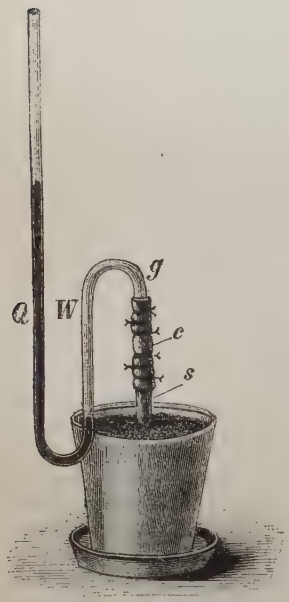


Fig. 12. The glass tube, *g*, is fixed by means of rubber tubing, *c*, to the stump of a *Dahlia*, *s*, and filled with water (*W*) and mercury (*Q*). (From the Bonn Textbook.)

Similar excretions occur elsewhere, not only directly from roots, but also from stems and branches, if these be cut off from the plant or if borings be made down to the woody cylinder. The phenomenon of 'weeping' or 'bleeding', as the gardener terms it, which occurs in spring-time, notably in the vine, when the stem is cut, is well known to every one. This phenomenon we may also designate by the term 'bleeding'. It has long been known that the sap is ejected from the plant often with very considerable force ('bleeding-pressure' or 'root-pressure'). Long ago, physiologists, e.g. HALES (1748), measured this pressure in essentially the same way as we do to-day. A glass tube, bent twice in the form of a U, is fitted to the stump of a root (Fig. 12) and filled with water just above the cut surface and then closed with mercury. By noting the height to which the mercury is driven in the open leg of the tube it is possible to estimate the amount of root-pressure.

Our next task must be to attempt a more exact estimate of the quantity, quality, and pressure of the sap expressed from such wounds.

On submitting the sap which has been collected to *analysis*, we find it to consist of water in which are always dissolved organic and inorganic substances although in very varying proportions. The extremely watery sap of the potato, the sunflower, and the vine, contains from 1 per cent. to 3 per cent. of solid in solution, of which two-thirds in the case of the vine, half in the sunflower, and one-third in the case of the potato is organic in its nature. The inorganic salts of the sap are exactly the same as those which we have already met with in the plant, while among the organic compounds, acids, proteids, and especially sugars occur. In concentrated sap the various sugars markedly predominate;

thus 1·4–1·9 per cent. of sugar has been found in the birch, in *Acer platanoides* 1·2–3·2 per cent., in *Acer saccharinum* 3·6 per cent., in *Agave americana* as much as 8·8 per cent. (SCHRÖDER, 1869).

The amount of sap excreted from day to day varies very considerably; sometimes it amounts only to a few drops, at other times it reaches several litres. It may not be without interest to present here, in tabular form, some of the maxima which have been observed (compare PFEFFER, Phys. I, p. 240; WIELER, 1893; MOLISCH, 1898).

Plant.	Observer.	Amount per day in litres.
Vitis aestivalis	(CLARK)	0·227
Vitis vinifera	(CANSTEIN)	1·0
Arenga saccharifera	(SEMLER)	3·0
	(MOLISCH)	4·6
Birch	(WIELER)	5·1
Ostrya	(CLARK)	5·6
Birch	(CLARK)	6·8
Agave americana	(HUMBOLDT)	7·5
Phoenix dactylifera	(SEMLER)	8·0–10·0 (*a*)
Musanga	(LECOMTE)	17·0 (*b*)
Caryota urens	(SEMLER)	50·0 (*a*)

(*a*) Whether SEMLER's results are to be depended upon must remain an open question. MOLISCH did not obtain such large amounts.

(*b*) Calculated on the results obtained during 10 hours.

The maximum outflow is not generally attained at once upon making the incision; usually there is at first a gradual increase in the amount excreted, followed later by decrease; we are unable, however, to attribute these variations to any definite external causes. BARANETSKY (1873) has shown this rise and fall very clearly in numerous tables which he has compiled, and MOLISCH (1898) also has given us the following numbers as applicable to *Arenga saccharifera*, where the amounts excreted in ccm. during fourteen days are indicated :—

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Day	440	500	1500	1400	1300	2050	1640	—	—	—	—	—	—	—
Night	675	1080	2175	2900	3350	1350	—	—	—	—	—	—	—	—
Total in 24 hours	1115	1580	3675	4300	4650	3400	—	1440	3600	2500	1140	700	175	0

From an examination of the table it will be seen that, taking into consideration the amount of sap excreted in the twenty-four hours, there is a gradual increase up to a maximum which is reached on the fifth day, followed by a decrease until the fourteenth day, when excretion ceases. It is also worthy of note that the decrease is by no means gradual and regular, but that a second maximum occurs on the ninth day. It is possible that the second rise may have been induced by *external* conditions, but in any case irregularities such as these exhibit themselves repeatedly and even more strikingly in experiments carried out in the laboratory under the most equable conditions, so that we are justified in assuming that the plant works irregularly owing to *internal* causes. There is yet another fact which the table teaches us, and that is, that the amount excreted by day is considerably less than that excreted by night.

Great variations also exhibit themselves in the *duration* of the outflow after the infliction of the wound. In palms the excretion often continues for two or three months; in *Arenga* it lasts for several years, and in *Agave americana*, whose sap (as in the case of palms) is used for the preparation of an alcoholic drink, the bleeding may continue, according to HUMBOLDT, for four or five months. The outflow continues for a shorter period in our indigenous trees (one month), and is most limited in small plants, where it may last for a few days only. Generally speaking, however, the lower limits of the duration of bleeding have not as yet been accurately determined. As a matter of fact, changes, which bring about an occlusion of the lumina of the vessels and a consequent

stoppage of the outflow, frequently occur on the cut surface, due not only to the activity of the plant itself, but also to the action of Bacteria. If a fresh surface be exposed, a renewal of the bleeding may not infrequently be observed, but this precaution has certainly not been taken in all experiments. Finally, it must be remembered that sooner or later excretion from a root-stump must come to an end, since the root itself dies when deprived of the nourishment which it would normally receive from the leaves.

Since, as we have seen, the duration of the bleeding and the amount of sap excreted daily show very marked variations, both specific and individual, it follows that the *amount* of sap excreted during the *entire bleeding period* must also vary to a very considerable extent. In the case of palms and *Agave*, where the bleeding is both excessive and long continued, the amounts which have been recorded are enormous. Thus *Agave* can, according to HUMBOLDT, give off in round numbers 1000 lit., a single axis of inflorescence of *Arenga*, according to SEMLER, produces 250 lit., although MOLISCH (1898) obtained from the same plant only 18–29 lit. Equal or even greater amounts have been obtained from our native trees. WIELER (1893) obtained, for example, 36 lit. from a birch tree in 8 days. Finally, with reference to the *pressure* exerted, WIELER (1892, 122) has given us a summary of a large number of determinations which he obtained on the subject of maximum pressures. At the present moment we cannot do more than quote a few selected examples from his tables. Low pressures occur in herbaceous plants; thus *Petunia* gives a pressure of 7 mm. of mercury, *Chenopodium*, 16 mm., *Ricinus*, 334 mm., *Urtica dioica*, 462 mm., the vine from 900–1100 mm., and finally the birch, 1390 mm., while CLARK obtained in *Betula lenta* as much as 1924 mm. These heights of the mercury column may be expressed in atmospheres, by saying that in *Ricinus* the pressure is equal to half an atmosphere, and in *Betula lenta* to two and a half atmospheres, and these appear to be the greatest pressures which can be attained as a result of normal root-bleeding. Under certain conditions, of which we shall have to speak presently, much higher pressures than these may be reached. Thus FIGDOR (1898) has recorded in the stems of certain tropical trees pressures of 6–8 atmospheres, and BÖHM (1892) and MOLISCH (1902) have obtained respectively pressures equal to 8·6 and 6·4 atmospheres in our native trees.

Just as the *amount* of sap excreted increases gradually, so also we find that the *pressure* does not reach a maximum all at once; at first it gradually rises and, later on, gradually falls. The periodic variations in the amount of outflow are indicative of correspondingly periodic fluctuations in pressure. In addition to daily and yearly fluctuations, irregular variations have also been observed. Although we must assume that the variations in the amount of outflow are due to the same causes as variations in pressure, still it does not follow that other and closer connexions exist between them. A large amount of water may be excreted though the pressure be quite low, and conversely high pressures may be accompanied by the excretion of very little water. This latter condition is exemplified in the case of the specially high pressures just cited (MOLISCH, 1902), where obviously only a few cells are concerned in the excretion of water, because these cells are separated off from their surroundings by layers of cells which are impermeable to water, and in such a state of affairs a high pressure may be readily attained. It is not at all improbable that there are individual cells in the ordinary root which allow water to filter through equally energetically; since other cells exposed to this pressure also permit water to pass, what we obtain by aid of the manometer is not the maximum activity of the individual cells but the resultant of secretion plus filtration.

It is particularly striking to note that when several manometers are connected with a stem at different levels (BRÜCKE, 1844), the decrease in pressure

from below upwards is by no means always regular, and further that the pressure variations in the individual manometers are often quite independent of each other. This phenomenon is explained especially by the fact that bleeding-pressure under consideration may exist not merely in the root, but also in a number of other places, in the stem, in the leaves, in the axis of inflorescence, and so on. Although the regions where bleeding occurs may be quite near to each other, it by no means follows that they are in uninterrupted communication with each other, and this condition is applicable to two nearly related regions in the xylem of a tree. Why intercommunication in this latter case is not unrestricted we shall discover when we consider the external conditions of bleeding.

The first and most general condition of bleeding is the occurrence of living cells in the neighbourhood of vessels. Death of the plant stops all further bleeding, and certain stimuli, which diminish its vitality without actually producing death, temporarily, at least, retard the phenomenon. For instance, WIELER (1893) brought the bleeding at once to a standstill by stopping the supply of oxygen, that is to say, by inhibiting respiration, and chloroform has the same effect. We may deduce, therefore, from these facts that bleeding is a vital phenomenon.

A second important condition of bleeding is that the cells which exhibit this phenomenon must be abundantly supplied with water, and this is effected by aiding absorption and retarding evaporation. The soil in which the roots lie must be well moistened, and the air kept saturated with the object of reducing transpiration. In our native trees bleeding may be observed most conveniently in early spring before the leaves come out, because, owing to the activity of the root, all the cells are saturated, and also because loss arising from transpiration is at a miminum. If, on the other hand, the tree be felled in summer, we find that, even after the soil has been well watered, not only does the cut surface exude no fluid, but that water poured upon it is at once greedily absorbed. But if, finally, a plentiful supply of water is collected by the root-system, then bleeding shows itself, and a positive root-pressure may be induced where previously a negative pressure, that is to say, a pressure less than that of the atmosphere existed.

A third condition of bleeding is a certain temperature, differing markedly of course in different plants. At 0° C. very few plants exhibit bleeding; others, such as the gourd, begin to bleed at 7°–9° C., whilst in all plants an increase in the amount of fluid given off accompanies an increase in temperature. Detailed investigations on this subject are, however, still wanting.

In addition to the three factors referred to above, there is yet another which is of some significance, viz. light, but this factor we need not discuss. Variations in these conditions are accompanied by corresponding variations in the amount of fluid excreted, and in the pressure exerted by the sap, and it would be natural to attribute the periodic fluctuations described above to the influence of these external factors. The researches of BARANETSKY appear to support this view, but those of later investigators scarcely confirm it.

The individual eccentricities are indeed quite incomprehensible. One of the most striking illustrations of this is given by WIELER, who has recorded diametrically opposite results arrived at from experimental observations made on two plants of *Alnus glutinosa*, which were not only of the same age but were similarly treated and studied under the same external conditions. While the one exhibited a minimum exudation of sap in the forenoon and a maximum in the afternoon, the other gave precisely converse results. When we recollect also that the same investigator was unable to demonstrate any periodicity at all in the birch, we can have no hesitation in concluding that we are still far from having reached a satisfactory explanation of this phenomenon.

To the factors above mentioned it is customary in many cases to add yet

another, viz. the wound inflicted on the plant. Very frequently the bleeding commences at once after the incision has been made, and then it is manifest that the vessels were already charged with water, and that the incision merely provided an opening for the exit of the fluid ; but in other cases it is not so, for the exudation may not begin to take place until some time after the wound has been made ; in such cases the actual wounding itself must have been the cause of the exudation. Until quite recently it was customary to attribute the exudation of sap containing large quantities of sugar from incisions made into the young axes of inflorescence of certain palms (*Cocos nucifera*, *Arenga saccharifera*, &c.) to the influence of *root-pressure*. According to MOLISCH (1898), however, no such root-pressure exists in these palms, and no sap exudes either from the stumps of the trees when felled, nor from auger holes bored in their stems; nor is there any excretion from the inflorescence itself, if only a simple incision be made in it. In *Cocos nucifera* bleeding first begins to take place after the apices of the inflorescence have been cut for several days in succession, and in *Arenga* a much greater effect is produced if, during the four or five weeks previous to flowering, repeated bruises be inflicted on the base of the boll by blows from a wooden hammer (a practice followed by the Malays); then, when the inflorescence is cut off, secretion begins at once. Nor are these altogether isolated observations. BÖHM (1892) was the first to observe the high pressures mentioned above (more than eight atmospheres), which might be obtained from our native trees by means of manometers fixed for a long period in auger holes bored in their stems ; and MOLISCH (1902) has drawn the conclusion that these pressures have nothing to do with *root-pressure*, seeing that the trees at the time of the experiment were *covered with leaves*, and when experimented upon with *fresh* auger holes gave, as indeed might have been expected, no positive pressure at all, sometimes even a negative one. Here also the secretion was, in the first instance, traceable to the actual wound itself and produced gradually and in its immediate vicinity. It originated, in all probability, from cells which arise or are stimulated to further growth in consequence of the wounding. At the same time various infiltration products enter the lumina of the vessels in the neighbourhood of the wound and render that part of the wood quite impermeable to water. It is quite evident, therefore, that a marked pressure may be produced purely locally, although a scarcity of water prevails in the immediate vicinity. MOLISCH speaks of such cases as local pressures, and it is very probable that *local* bleeding may take place not merely in palms and other trees above mentioned, but generally in all cases of amputated branches and leaves, where the existence of bleeding has been established (PITRA, 1878), whilst what may be termed *normal* bleeding is associated, perhaps, with the root only.

That bleeding, i. e. the unilateral excretion of water from parenchyma into vessels, is an osmotic phenomenon, has been generally accepted since the time of DUTROCHET. In order to obtain an explanation of this unilateral excretion, however, we must now study examples of plants which exude sap of very weak concentration, and we may assume that the cells concerned are lined by protoplasm which is completely impermeable to the substances dissolved in the vacuole. Then comes the question, how can a unilateral expression of water take place from such a turgescent cell ? In an ordinary cell the all-round endosmosis of water is balanced by the exosmosis caused by the pressure from within ; as in one place more water will exude than enter, so in another place more water will flow in than flow out. In order to explain the varying behaviour of the different sides of the cell it has been the custom hitherto to ascribe it to the different qualities of the plasmatic membrane, since it was believed that the degree of osmotic pressure was dependent on the character of the plasmatic membrane. If one half of the cell possesses

a membrane which gives a lower osmotic pressure than the other half, then on that side undoubtedly there will be a continuous outflow of water. This view has been, however, demonstrated to be fundamentally erroneous. As PFEFFER (1890, p. 303) has shown, and as is itself obvious from the kinetic theory of osmotic pressure, the degree of pressure depends only on the number of molecules plus free ions, and not in the least on the quality of the protoplasm; for a precipitation membrane, no matter how variable it may be, chemically and physically, no matter whether it be thin or thick, must always give the same pressure *so long as it is impermeable.* PFEFFER'S (1877) explanation, on the other hand, may be looked upon as the physically correct one. If at different points in the cell different degrees of concentration of the cell-sap arise, the inflow on the side of greater concentration must still exceed the outflow at the moment when equilibrium between the two has been already established on the other side; the result is a unilateral outflow of water under a pressure corresponding to the difference in concentration on the two sides of the cell. Such a difference of concentration cannot obtain in physical experiments, since, in consequence of diffusion, readjustment of the balance must necessarily take place. If it occur and be also maintained in the plant, however, an expenditure of energy is clearly essential, such as the living cell can always furnish, but which a physical apparatus (PFEFFER'S osmotic cell) does not possess. This is quite in accordance with what we have already learned, viz. that unilateral secretion of water is at once inhibited by the withdrawal of oxygen or by the action of chloroform, whereby the cells are transformed into non-living, purely physical pieces of apparatus.

An entirely different theory as to the cause of unilateral excretion has been advanced by GODLEWSKI (1884). He postulates rhythmic and continuous variations in osmotic pressure, during which the osmotically active substance is always breaking down and as constantly being built up afresh. At each lowering of the osmotic pressure there is an excretion of water, owing to the contraction of the elastically distended wall, and should these contractions follow each other at longer or shorter intervals, it may be said that the cell exhibits pulsations. Although there is much to be said in favour of this idea, looked at by itself, its absolute correctness may be called in question since, in the first place, there seems to be no good reason why the water, in these pulsations, should always be forced out on one side only, and, in the second place, there would seem to be every reason for the re-absorption of the water secreted immediately on the re-formation of the osmotically active substance.

A third explanation which has been advanced lays special emphasis on the *concentration* of the sap. The assumption is that outside the cell which is excreting water there arises, either in its membrane or in the membrane of the vessel, an osmotically active substance which withdraws water by osmotic suction from the cell; the cell itself would, according to this view, act in an entirely passive manner during the process. That such a phenomenon does take place in nectaries has been definitely proved, but whether it also plays a part in the process of bleeding is very doubtful. WIELER has estimated that the osmotic pressure of bleeding may rise in the birch to two and a half atmospheres, so that it is quite legitimate to consider the pressure as due in this case to osmotic action; and it is still more feasible to make this assumption in the case of the much more sugary sap of *Acer*, *Agave*, and Palmaceae. But it is quite impossible to conceive that *bleeding-pressures in general* are in this sense osmotic, since no definite relation can be observed between the amount of the pressure and the concentration of the sap. In the vine, for example, high pressures may be accompanied by a low concentration of sap. Moreover, WIELER has conducted a series of experiments on the subject, and has found that when he allowed osmotically active solutions to soak into the vessels of the root-stump the bleeding was not increased at all.

Then again, it is very difficult to believe that an amount of sugar as great as that which may be obtained from palms, *Agave*, and other plants, comes from the *cell-walls*; obviously it must have been formed in the *interior of the cell*, and, inasmuch as it passes through the protoplasm in order to reach the exterior, the plasma cannot be so impermeable as we have hitherto been led to believe. So soon as we postulate a unilateral permeability for the protoplasm, however, the conditions necessary for unilateral excretion of sap are fulfilled, because in this way a permanent difference in the concentration of cell-sap on the different sides of the cell is established. If the *qualitative difference* in the plasmatic membrane at different situations in the cell consists in its being impermeable on one side, on the other partially so, unilateral excretion of water becomes possible; but the fluid that filters through is always *cell-sap*, however dilute it may be, and never pure water.

We are not in a position at present to advance conclusive evidence in favour of one rather than another of the explanations which have been put forward to account for bleeding; but after due consideration we are inclined to favour the view that bleeding is to be accounted for by differences in the concentration of cell-sap on two sides of the cell, differences which are often produced and maintained by expenditure of energy on the part of the cell itself, often also by unilateral permeability of the protoplasm.

Bleeding, so far as we have considered it, though of the greatest importance to the plant physiologist, cannot be regarded in any other light than as an injurious and even pathological process to the plant itself. No matter whether it be pure water or a concentrated sugar solution which escapes from the wound, the plant is always suffering a loss of material without receiving any compensation. In the *uninjured* plant we find that an excretion of water takes place under pressure into the vascular strand. It is true we are, as a rule, unable to prove this fact directly, since the exudation of water is manifested only after the infliction of the wound; but the fact that at certain times and in certain plants excretion of water takes place immediately upon the cutting off of a branch, proves indubitably that a bleeding pressure exists in the plant, although no incision has been made. T. Hartig (1853, 1862) has already observed that in spring, before the unfolding of the leaves, sap exudes from the buds of the hornbeam and other trees, although no lesions are visible. Strasburger (1891, p. 840) has also more recently demonstrated that this extravasation of drops is a *result* of *bleeding pressure*, and that drops exude from the apices of the leaves of the previous year, whose cuticle has been burst open. This feature is by no means an annual one, nor is it observable in all hornbeams, hence we must conclude that an especially high pressure is necessary, not only, in the first place, to force the water up to the apices of the branches, but also, in the second place, to overcome the opposition which the leaf apices present to the outflow.

What is in arboreal plants the exception is the rule in many herbaceous plants; for under favourable conditions, especially excessive dampness of the soil and reduced transpiration, conditions found during the night, water is forced into the whole vascular system of such plants, mainly through the activity of the root, with such force that an exudation of drops takes place wherever opposition to filtration is reduced. A noteworthy example of this phenomenon is furnished by the leaves of *Colocasia antiquorum*, and other Aroidaceae, e. g. *Remusatia vivipara* show it also. The drops in these cases exude exclusively from the apices of the leaves and succeed each other very rapidly. In *Colocasia*, Duchartre (1859) counted ten to fifteen, and, in extreme cases, as many as thirty, drops falling from the leaf-apex every minute. Each drop, moreover, was formed from the coalescence of five to six smaller drops expressed simultaneously from the leaf, so that the leaf gave off a maximum of 180 drops per minute, or three per second, and the total quantity of fluid which could be

collected in the course of a single night amounted, generally speaking, to about 10 g. and might reach as much as 22 g. The sap contains very few substances in solution, for BERTHELOT, who at DUCHARTRE'S suggestion undertook an analysis of the fluid, was unable to do more than demonstrate the presence of the merest traces of organic and inorganic materials in as much as 400 g.; we may, therefore, speak of the fluid, for our present purpose, as practically pure water.

The same phenomenon, differing from that seen in *Colocasia* only in degree, occurs in many of our indigenous and cultivated plants. After a warm night, small drops resembling dew are to be seen at the apices of leaves, on the leaf teeth, and, more rarely, on other parts of the leaf. It is quite easy to show that these are in no sense dewdrops, but are the result of the activity of the plant itself, for they occur frequently only on the young leaves, while there is no reason why dewdrops should not appear on the older leaves as well. The drops increase gradually in size, fall off, and are once more replaced, but they never aggregate into such quantities of fluid as are given off by *Colocasia*. Well-known examples of the excretion of drops are furnished by the leaf apices of grasses, the leaf teeth of *Fuchsia*, *Alchemilla*, *Brassica*, and the potato; while *Tropaeolum* and many Urticaceae and Moraceae give off water not only from the edges of their leaves but from the surfaces as well.

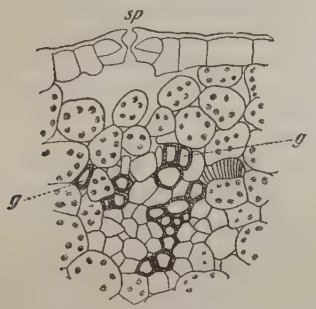


Fig. 13. Water stoma of *Vicia faba* in section. *sp*, the stoma; *g*, vessels abutting on the intercellular spaces. (After HABERLANDT, 1895, pl. 3.)

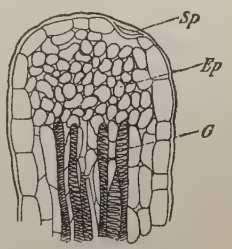


Fig. 14. Longitudinal section through a leaf tooth of *Primula sinensis*. *Sp*, stoma; *Ep*, epithem; *G*, vessels. (After HABERLANDT, 1895, pl. 4.)

This excretion of water is effected by special organs, the so-called 'hydathodes'. These differ, as a rule, from ordinary stomata in being larger and in having immobile guard-cells; for these reasons they have received the special name of 'water-stomata'. They occur singly or in groups in the situations where excretion of water takes place, and it is through them that the water escapes which has accumulated in the underlying space corresponding to the respiratory cavity of the ordinary stoma (compare p. 37). Usually the ends of the vascular bundles only lie in close relation to these cavities. In the simplest cases (grasses and *Vicia sepium*, Fig. 13) the ultimate tracheids run immediately under the respiratory cavity, and frequently small superficial tracheids border directly on this large intercellular space, or are separated from it only by loosely arranged parenchymatous tissue; the parenchyma which immediately invests the vascular elements is practically destitute of intercellular spaces. In the more highly developed organs which occur in *Fuchsia* and *Primula* (Fig. 14), the tracheids open out at the ends of the bundle in a brush-like manner, and the spaces between the individual tracheids and also the not inconsiderable gap between the ends of the bundles and the water-stomata are filled by parenchymatous cells. This parenchyma (epithem: *Ep*, Fig. 14) is composed of cells which are much smaller than those of the mesophyll, and is not infrequently deliminated from it by a cuticularized sheath.

There are marked intercellular spaces between these cells, spaces which appear to be always full of water, even though no secretion is taking place.

In the plants which have been referred to (the Urticaceae and Artocarpaceae, perhaps, excepted) the epithem plays the part of a filter. It is only when a positive pressure has been developed in the vessels that an excretion of water begins, and naturally this excretion takes place at the region of least resistance; the water leaves the tracheids, enters the intercellular spaces of the epithem, and escapes thence to the exterior. This process is facilitated by the same external factors which aid in the raising of the bleeding-pressure and the exudation of drops, and hence we are able to induce this exudation by elevating the temperature sufficiently, and by moistening the soil and the air, at times when, under normal conditions, the plant exhibits no such phenomena. It has long since been demonstrated that the phenomenon we have been considering is due to the pressure of sap in the vascular system, and that if water be forced into the cut end of a branch by means of a column of mercury, say 20 cm. in height, an excretion of drops may be at once induced. If instead of employing water we use a watery solution of a colouring matter incapable of penetrating the protoplasm (MOLISCH, 1880), the coloured solution is observed to issue from the leaf teeth unaltered, indicating that the epithem, in so far as it consists of living cells, takes no part in the filtration. HABERLANDT (1894) has demonstrated this fact perhaps even more clearly by killing the epithem by means of corrosive sublimate, and showing that the excretion of water continues notwithstanding. On the other hand, he showed that in the Artocarpaceae and Urticaceae the excretion of water ceased on the death of the epithem cells, and for that reason he attributed to them in these cases a special capacity for actively excreting water. This has not, however, been confirmed by other investigators (e. g. SPANJER, 1898), so that at present it may be considered as doubtful whether two types of epithem occur in plants, a *filtering* epithem and an *actively secreting* epithem.

The existence of 'actively' secreting *hydathodes* is, however, well established. Many epidermal cells, i. e. such as grow out into epidermal hairs, are known as hydathodes. Examples of these occur in the hairs found in the hollow leaves of *Lathraea* (GOEBEL, 1897, HABERLANDT, 1897), and especially in the secretory hairs of many insectivorous plants. The fact that there is, as a rule, no vascular bundle directly connected with these hairs renders it apparently impossible that the excretion of water can take place in these cases by simple filtration. It is obvious that these hairs obtain their water supply osmotically, and that their cells are possessed of an osmotic activity comparable in all respects to that exhibited by those parenchymatous cells of the root which excrete water into the vessels in that organ. The difference is only one of situation, and we will describe as *water glands* all organs which exhibit unilateral excretion of water.

Owing to its chemical characters we have previously described the sap, which exudes from the leaf apices of *Colocasia*, simply as 'water'. Although this nomenclature may be appropriate in the case of *Colocasia*, it is by no means always so in the case of other hydathodes, whether these be active or passive. Very frequently the fluid contains carbonate of lime, which makes itself apparent in the form of separate crystals or complete incrustations, after the evaporation of the water. Examples are well known to occur in the filtering hydathodes of species of Saxifragaceae, where the occurrence of scales of carbonate of lime in the depressions into which the water filters is well known. The hairs of *Lathraea*, as already mentioned, are especially good illustrations of actively secreting hydathodes. In these cases the protoplasm must undoubtedly be permeable to lime salts, just as it is permeable in other cases to other salts. Thus in many Tamaricaceae and Plumbaginaceae peculiar glands are found, by the activity of which these plants become covered over with a greyish

incrustation of a saline nature which has the power of absorbing hygroscopic water (MARLOTH, 1887). Hairs which give off *acid* secretions have frequently been described, e. g. by STAHL (1888), in *Cicer arietinum*, *Circaea lutetiana*, and *Epilobium hirsutum*, while they are widely distributed in insectivorous plants, where, in addition to an acid, a proteolytic enzyme is also present (compare Lecture XV). In many Fungi also, both unicellular types, such as *Pilobolus*, and multicellular (*Penicillium*, *Peziza sclerotiorum*, *Merulius lachrymans*, *Clavi-ceps purpurea*, &c.), drops of fluid have been observed to be excreted, which on analysis are often found to be rich in organic materials, such as oxalic acid and sugars of various kinds. The excretion of sugar is a frequent phenomenon in the higher plants in the nectaries which, although they occur more especially in the floral organs, are found on vegetative organs as well. There is practically no difference in fundamental character between the water glands we have hitherto been discussing and these nectariferous cells, so that, beyond referring to the literature on the subject (WILSON, 1881, PFEFFER, 1892, BÜSGEN, 1891, HAUPT, 1902) we need not go further into the question, more especially as it would lead us too far away from our present subject.

The general survey of all the phenomena, which we may group together under the head of *exudation of water*, shows us how varied these processes are, looked at from a purely physiological point of view; it can scarcely be expected, therefore, that they will all fulfil the same or even similar functions in the plant. The significance of nectaries is best known; they induce insects to visit the plant, and thus in very many cases facilitate the transport of pollen to the stigma. Quite as familiar is the significance of the secretion which occurs in insectivorous plants, a secretion which we shall have to study in detail later on. In this case the secretion aids in the digestion of captured insects, and in most cases indeed it is produced only when the opportunity for such digestion occurs.

It is not so easy to ascribe biological meanings to some of the other secretions mentioned in the preceding pages. If, along with the water, large amounts of common salt or of carbonate of lime are given off, we may assume that the plant is in this way ridding itself of superfluous or injurious substances. The lime, it is true, may be found in many plants deposited *within* the body either as oxalate in the cell-cavity, or as carbonate in the cell-wall; but it would scarcely be correct to conclude that when it is excreted to the exterior its actual excretion serves any special purpose, the more so as *silicic acid* does not lend itself to such excretion. Common salt, on the other hand, as we shall have occasion to see later on, may cause direct injury to the plant, and since, in its case, the precipitation of the fundamentally potent element chlorine cannot be effected by the formation of an insoluble compound, there is no difficulty in apprehending the advantage the plant obtains from its definite excretion.

It is quite otherwise with the excretion of pure, or almost pure water. In this case the *actual removal* of the *water* as such from the plant cannot be the object to be attained, any more than the removal of water vapour can be the most important aim in the process of transpiration. If we have rightly apprehended the significance to be attached to the rapid movement of nutritive salts, it will be at once apparent that this exudation of water in the form of drops must be regarded as a process which *takes the place of transpiration* in situations where transpiration is, for other reasons, out of the question. *Continuous* transpiration in aquatic plants is impossible, and in their case excretion of liquid water has been often observed (WEINROWSKY, 1899). [Compare also POND, 1905 (Biological relations of aquatic plants to the substratum. U.S.A. Fish Comm. Reports, 1903. Washington, 1905).] Moreover, transpiration will be retarded temporarily in many land plants both during the night and in the early morning, owing to the saturation of the air. It is just at these times that the exudation of drops is conspicuous in such plants.

Only plants that have a great development of water tissue can afford to be lavish in their use of this water. Amongst our native plants, save the willow, herbaceous forms only exhibit excretions of water when in full leaf, but in tropical countries, where the water supply is abundant, it is especially noticeable, e. g. in the long-stemmed lianas. If the excretion of fluid water by hydathodes be rendered impossible in appropriate ways, the injection of the intercellular spaces of the leaf takes place. In spite of the statements of LEPESCHKIN (1902) that the activity of leaves is not affected by the infiltration of water into them, we cannot but believe that the prevention of such an infiltration by the activity of hydathodes must always be of service.

Since probably all hydathodes are capable, under certain circumstances, of *absorbing* water, they may be of service in this respect also.

In cases where an actual excretion is not attained, bleeding-pressure may be useful to the plant; thus it is possible to demonstrate that such pressure aids in the unfolding of buds in spring.

The chief problem left for us now to consider is *how does root-pressure aid the ascent of sap in the plant? We have already established the fact that by its means water is pumped into the vessels*; whether it aids to any extent in the transport of water the next lecture will show.

Bibliography to Lecture V.

BARANETZKY. 1873. Abh. Naturf. Gesell. Halle, 13, 3.
BÖHM. 1892. Ber. d. bot. Gesell. 10, 539.
BRÜCKE. 1844. Annalen d. Physik u. Chemie, 63, 193. Ostwald's Klassiker, Nr. 95.
BÜSGEN. 1891. Der Honigtau. (Jen. Ztschr. f. Naturw.)
DUCHARTRE. 1859. Ann. sc. nat. IV, 12, 267.
ELFVING. 1882. Bot. Ztg. 40, 714.
ERRERA. 1886. Bot. Ztg. 42, 16.
FIGDOR. 1898. Sitzungsber. Wien. Akad. 107, I, 641.
GODLEWSKI. 1884. Jahrb. f. wiss. Bot. 15, 602.
GOEBEL. 1897. Flora, 83, 444.
HABERLANDT. 1894 and 1895. Sitzungsber. Wien. Akad., M/N Kl., 103, I, 489; 104, I, 55.
HABERLANDT. 1897. Jahrb. f. wiss. Bot. 30, 511.
HALES. 1748. Statik der Gewächse. Halle.
HARTIG, T. 1853. Bot. Ztg. 11, 478.
HARTIG, T. 1862. Ibid. 20, 85.
HAUPT. 1902. Flora, 90, 1.
HOFMEISTER. 1862. Flora, 45, 97.
HUMBOLDT, cited in Meyen's Pflanzenphysiologie, 2, 85.
KOHL. 1886. Die Transpiration, etc. Braunschweig.
LEPESCHKIN. 1902. Flora, 90, 42.
MARLOTH. 1887. Ber. d. bot. Gesell. 5, 319.
MOLISCH. 1898. Sitzungsber. Wien. Akad., M/N Kl., 107, I, 1247.
MOLISCH. 1902. Bot. Ztg. 60, 45.
MOLL. 1880. Verslagen u. Meded. Akad. d. Wet. Natuurk., R. 2, Deel 15.
PFEFFER. 1877. Osmotische Untersuchungen. Leipzig.
PFEFFER. 1890. Plasmahaut u. Vacuolen. Abh. Kgl. Gesell. d. Wiss. Leipzig, 16.
PFEFFER. 1892. Studien z. Energetik. Abh. Kgl. Gesell. d. Wiss. Leipzig, 18.
PITRA. 1878. Jahrb. f. wiss. Bot. 11, 437.
REINKE. 1902. Ber. d. bot. Gesell. 20 (97).
SACHS, J. 1879. Arb. d. Würzburg. Instituts, 2, 291.
SCHRÖDER. 1869. Jahrb. f. wiss. Bot. 7, 261.
SEMLER. 1886. Handbuch d. trop. Agrikultur, 1. Wismar.
SPANJER. 1898. Bot. Ztg. 56, 35 (compare Bot. Ztg. 56, II, 177, 241, 315).
STAHL. 1888. Pflanzen u. Schnecken (Jen. Ztschr. f. Naturw.), p. 42.
STRASBURGER. 1891. Bau u. Verrichtungen d. Leitungsbahnen. Jena.
TRÉCUL. 1855. Annal. sc. nat. IV, 3, 343.
VESQUE. 1883. Compt. rend. 97.

WEINROWSKY. 1899. Fünfstück's Beitr. 3.
WESTERMAIER. 1884. Sitzungsber. Berlin. Akad., p. 1110.
WIELER. 1893. Cohn's Beitr. z. Biol. 6, 1.
WILSON. 1881. Unters. aus d. bot. Inst. Tübingen, 1, 8.

LECTURE VI

THE CONDUCTION OF WATER. II

HAVING seen how water enters the vessels, we have now to inquire as to the forces which bring about its ascent to the tops of lofty trees. In order to obtain some idea of the amount and direction of these forces it will be necessary for us first of all to get a clear conception as to the course, the quantity, and the rapidity of carriage of the water, and of the height to which it is carried. As to the *direction* followed by the water, there is no question, at least in all ordinary cases, viz. from below upwards, from the absorbing root to the transpiring leaf. It is important to note, however, that a current may be formed in the *reverse* direction, there being no special appliances in the interior of the vessels for guiding the flow in one direction only. Indeed the older physiologists who studied the subject held strongly that water could move as easily from the apices of the branches down to the root as in the normal direction. More recently, TH. HARTIG (1861) showed that solutions of certain substances could travel through the wood of felled trees and of isolated branches in the reverse direction, if the upper parts were submerged instead of the lower. Much the most convincing, however, was the experiment performed by STRASBURGER on the beech (1891, p. 938). He employed a stem which had fused high up with a neighbouring stem, the latter being abundantly provided with leafy branches right down to the ground. He severed this stem at its lower end so that both it and its branches were entirely dependent on the water which the other stem had absorbed, although in order to reach the lowest branches the water had to flow from above downwards. These branches, however, remained quite fresh for several years (STRASBURGER, 1893). The experiment showed, in addition, that the *amount* of water which flowed in the reverse direction to the normal was amply sufficient to maintain turgidity in the leaves of the lowest branches.

The *amount* of water transpired gives us some indication as to the quantity which normally travels up a tree trunk. A glance at the flaccid leaves of plants, as they appear in the evening of a hot summer day, shows clearly that more water is evaporated from the leaves than is carried up to them. But since the leaves by the following morning have again become rigid, it follows that they must have recouped themselves during the night for what they have lost by day. Generally speaking, it may be said that the amount of water transpired during the twenty-four hours is roughly equivalent to what is lifted in the same time. These amounts might be found identical were it possible to prove that the amount of water contained in the wood showed no variations, but that is not very probable. We owe our knowledge of the amount of water contained in tree trunks at different seasons of the year to the laborious researches of R. HARTIG (1882). Unfortunately each determination necessitated the felling of the whole tree, so that it was impossible to estimate how much of the result was due to individual variations and how much to the season of the year. When one remembers that research has shown that, in the case of the Scotch pine, 50 per cent. of the entire volume of the wood, according to one estimate, and only 25 per cent. according to another, was water, we are compelled to assume the existence of variations in water capacity in each stem, and to look

on the wood as an immense water reservoir, which in times of abundance (e. g. when transpiration is retarded) is full, and in times of scarcity (e. g. under continuous drought) is depleted. For this reason it is impossible to draw any reliable conclusions as to the amount of water carried by the stem from a calculation of the amount of transpiration taking place during a single day.

The amount of transpiration taking place in a tree can be estimated only with an approximation to accuracy, and even though we knew the number and average diameter of the vessels we would still be ignorant as to how many of them took part in the transport of water. The fact is that the whole of the wood in the transverse section of a tree behaves by no means uniformly in this respect. To begin with, all the so-called heart wood may be neglected, because the lumina of the vessels in this region are quite unable to conduct water owing to thyloses and other obstructions. In typical heart wood trees, such as, for example, the oak, an incision, sufficiently deep to interrupt the continuity of the sap wood, is enough to arrest the conduction of water. Indeed in many cases this layer of sap wood is so thin that mere ringing of the bark impairs, and even interrupts, the supply of water (*Rhus typhina*). On the other hand, there are trees which form scarcely any heart wood and whose older wood retains the power of conduction. To this class the lime belongs, and it will be remembered how it was shown in the last lecture that this plant is capable of withstanding for many years the injurious effects of ringing. In an ordinary heart wood tree, on the other hand, the sap wood beneath the place where the operation of ringing has been performed is rapidly destroyed and the apex withers. If a sharp line of demarcation exists between the heart and sap wood, and if we assume, on the one hand, that the whole of the sap wood is of equal value for conducting purposes and that the whole of the heart wood is valueless, still only approximate estimates of the area of the part of the wood actually concerned in the transport of water can be given. The transition between heart and sap wood is, however, quite gradual, and not infrequently the lumina of the vessels formed in the second and third years of growth are blocked up by thyloses (*Robinia*, WIELER, 1888). Generally speaking, the youngest annual ring is the most effective water conduit, and the capacity for conducting water gradually decreases as the centre is approached, where possibly the tissue plays the part of a *water reservoir* only.

An estimate of the *rapidity of movement* of water based on the transverse area of the conducting system and on the amount of water flowing through it is, likewise, out of the question. A rough idea of the amount, however, may be arrived at by employing the method perfected by SACHS (1878), but based on the experiments of MCNAB (1871) and PFITZER (1877). SACHS allowed the plant to absorb a solution of lithium nitrate through the root, after finding that this substance penetrated the protoplasm very rapidly without doing it any injury and passed into the vessels as quickly as did the water itself. Since lithium does not as a rule occur in the plant, and further, since the minutest trace of that substance can readily be detected by the spectroscope, the rate of its ascent could be easily determined. Some of the data with regard to the hourly ascents which SACHS obtained are summarized in the following table :—

Acacia lophantha	(aver.) 154·0 cm.
Nicotiana tabacum	118·0 „
Musa sapientum	(aver.) 100·0 „
Cucurbita pepo	63·0 „
Podocarpus macrophylla	18·7 „

It must not be supposed that these numbers indicate either the highest or the lowest extremes of rapidity with which the fluid may ascend the tree.

The *height* to which water must in the long run be raised is, in the case of some plants, very remarkable. *Eucalyptus amygdalina*, with a height of

140–152 m., and *Sequoia gigantea*, with a height of 79–142 m., stand out as giants in the plant world; *Abies pectinata* (75 m.), *Picea excelsa* (60 m.), *Fagus sylvatica* (44 m.), *Platanus* and *Fraxinus* (30 m.), may also be cited as examples of trees of great height.

These observations, unfortunately, lead us to a very perplexing result. The ascent of water cannot be treated merely as a physical problem, for some of the most critical data required for the solution of the question are wanting; nor is it possible for the moment to speak of a *theory* of the movement of water in the plant, since not one of the numerous investigations to which the problem has given rise has up till now provided us with an estimate, proving that the amount of water raised by the process assumed is actually commensurate with that raised in the plant. So long as such *quantitative* evidence is wanting we may, in our opinion, speak of hypotheses only, not of theories.

With these preliminary remarks we may now attempt to study more closely the nature of the forces which operate in the ascent of sap. We are unable to do more than present a critical analysis, which itself makes no pretension at completeness. An historical enumeration of the voluminous researches which have been made on this problem since the time of HALES would be indeed of interest, but for this we cannot afford space (compare COPELAND, 1902).

In the first place we might imagine that the water was forced up by *root-pressure*, and it is apparent that bleeding-pressure must operate to some extent in this way. But the question at once arises as to whether this force is sufficient to force water to the tree top, and whether the amount of water supplied by the root is approximately enough to replace what is lost in transpiration. Some experiments carried out by SACHS (1873) are worthy of consideration on this latter point. He compared the amount of sap given off in a definite time from the root of a herbaceous plant with the amount sucked up by a shoot whose cut end had been submerged. A root-stock of *Nicotiana latissima* gave off about 16 ccm. of sap in five days, but its shoot absorbed 200 ccm. A similar disparity was exhibited in other cases also. Further, it is very improbable that the secretory capacity of the root is sufficient of itself to compensate for the loss of water due to transpiration. It must be remembered, however, that variations may be set up in the root owing to the amputation of the shoot. [Compare also DARBISHIRE, 1905 (Bot. Gaz. 39, 356).] So far as the pressure which causes bleeding is concerned (apart altogether from local pressures, which must obviously be entirely disregarded in this connexion), we may accept the statement of WIELER (1893) as indicating the extreme pressure which has been demonstrated with certainty. WIELER found that in the case of the birch a pressure of 139 cm. of mercury, or about two atmospheres, was developed. If we disregard the frictional resistance presented by the vessels, such a pressure might force water up to a height of 20 m., but it could not raise water to the top of a growing tree 25 m. high. Let us take another example. The silver fir may reach a height of 75 m., and if water is to be elevated to the top of such a tree by means of bleeding-pressure only, that pressure must amount to at least seven and a half atmospheres. Bleeding-pressure, however, in all Coniferae is extremely feeble, as very many estimates clearly prove. HOFMEISTER (1862), indeed, states that, as a general rule, Coniferae do not exhibit the phenomenon of bleeding; and though WIELER (1893), at a later date, was able to show that bleeding could be demonstrated in these plants, still it is impossible to draw any other conclusion than that it was quite insignificant in amount. Quite apart from Coniferae there are many other plants whose maximum root-pressure is quite inadequate to bring about the filling of the vessels of the leaves and branch apices. Thus, according to WIELER'S statements (1893, p. 122), the root-pressure in *Morus* reaches only to 12 mm., in *Fraxinus* to 21 mm., and in *Acer pseudoplatanus* from 169–313 mm. of

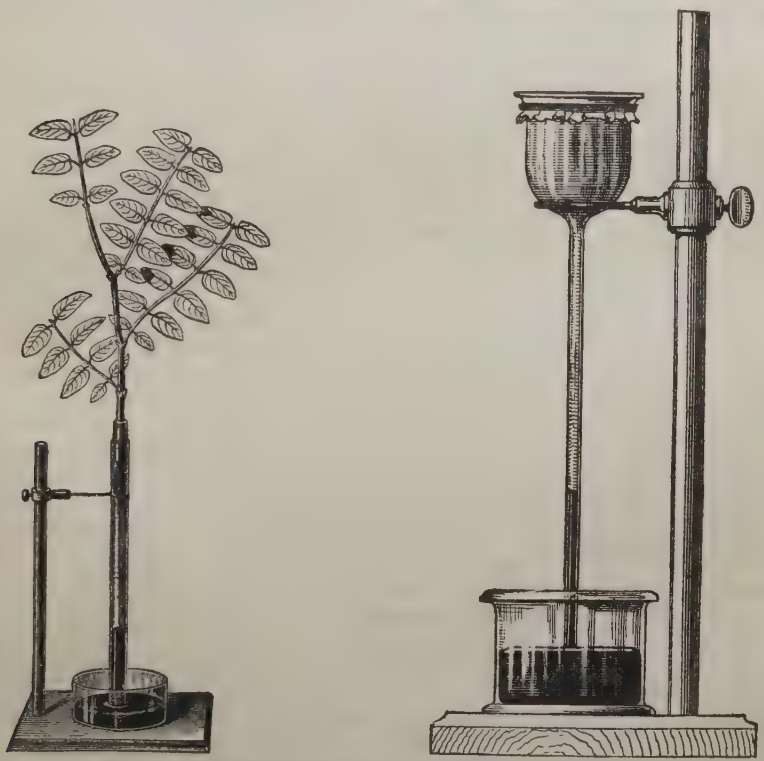
mercury. It must further be specially remembered that this *maximum* pressure shows itself, in general, in all our native plants only under conditions when the evaporation of water is much reduced. Bleeding may be observed in the birch and vine in springtime, *before* the appearance of the leaves, but in the middle of summer special precautions have to be taken to retard transpiration before the phenomenon can be demonstrated. When transpiration is most vigorous no water exudes from the stump of a felled tree, indeed it is greedily absorbed, and if a manometer be fixed laterally in the wood of a leafy stem, it shows a negative rather than a positive pressure, that is to say, a pressure in the interior of the stem less than that of the atmosphere. This negative pressure, to which we have previously drawn attention, is especially well seen in leafy branches, so that it is obvious that the bleeding-pressure which makes itself apparent in twigs, branches, and in the stem itself, does not exist at all during transpiration, or is so inconspicuous that it is not worth considering as a means whereby water may be supplied to the leaves. This tallies with V. HÖHNEL's observations (1879) on herbaceous plants, for he observed that grasses gave off water in the form of drops in the morning owing to the repletion of their vessels, and exhibited a conspicuous negative pressure in the afternoon when transpiration became vigorous. For these reasons it is impossible to ascribe to bleeding-pressure any fundamental significance *apropos* of the conduction of water, although, perhaps, when it does occur, it may to some extent aid in the ascent.

If, then, water be not forced up by the agency of a pressure acting from *below*, we are compelled to cast about for a force which must have its seat of activity at the *upper end* of the plant, and which is capable of *sucking* the water up. We have already learned of the existence of such a force, viz. transpiration; our experiments with the potometer made this clear to us, but it is possible to demonstrate its existence much more definitely if we fix a transpiring shoot, air-tight, in a long glass tube, fill the tube with water, and stand it vertically with its lower end immersed in mercury (Fig. 15). The branch continues to absorb water by its cut surface, and mercury rises to take its place in proportion as the water disappears from the tube. The height to which the mercury ascends serves as a direct indication of the force exerted by the suction. This sucking process is, however, a purely physical phenomenon, and for that reason it will be advisable for us to study it in the first instance with the aid of a physical apparatus. We employ for this purpose a thistle tube, closed at the expanded end by parchment and filled with water (Fig. 16), and with its lower narrow end plunged into mercury. The parchment loses its imbibed water by evaporation and, in consequence, sucks water out of the tube. This water is in turn replaced by mercury. Since the atmospheric pressure on the outside of the membrane exceeds the atmospheric pressure on the inside by the weight of the column of water and mercury, the air easily passes through the parchment into the interior and occupies the space between the membrane and the water, and any further ascent of mercury ceases. For this reason, ASKENASY (1895) replaced the parchment by a block of gypsum, which, when wet, is less permeable to air. Since the pressure in the interior of the apparatus becomes less and less as the mercury rises higher and higher, the air escapes from the water just as in an air-pump and stops any further ascent. If one uses boiled water, however, very considerable heights are obtained. In ASKENASY'S (1896) experiments, for example, the mercury reached a height of 82 cm. with the barometer standing at 76·2 cm., and, in another case, 89 cm., when the atmospheric pressure was 75·3 cm. It is obvious that still higher values could have been obtained had the gypsum cork been able to resist the passage of air entirely.

These heights reached by the mercury, exceeding as they do that of the

mercury in the barometer, are at first sight surprising, because they appear to contradict the lessons learnt from the Torricellian vacuum. How can these facts be explained, and such heights be theoretically accounted for ?

By employing an air-pump in place of the evaporating block of gypsum, and filling the glass tube with *air* from the beginning, it would certainly be possible to produce a vacuum on attaining a height comparable to that produced by atmospheric pressure. In order to obtain this result in our experiment, however, the adhesion between water and plaster of Paris, between the water and the wall of the tube, as well as the cohesion of the water particles themselves, must, first of all, be overcome. It is well enough known that the force



Figs. 15, 16. From DETMER's Smaller Practical Physiology (Figs. 77 and 78).

of adhesion is very great, but, taking as a basis the older physical experiments on the subject, it is quite obvious that we have considerably underestimated the force with which the water particles cohere. ASKENASY and DIXON have performed a very great service in showing how immense that force really is. Detailed estimates of the force of cohesion are as yet wanting, still we may for the present be content with the results arrived at by DIXON and JOLY (1895, b, p. 570), according to whom a pull equivalent to at least seven atmospheres is necessary to tear asunder a column of water. In all probability this is an under- rather than an over-estimate of the force of cohesion. If it can only be arranged that no air passes into the gypsum, a column of water 70 m., or even more, or a column of mercury, 5½ m., can be held in suspension by an evaporating block of gypsum. According to the detailed statements of REINGANUM (1896) and NERNST (1900) columns of water of considerably greater length must be supported by transpiration.

If in place of the block of gypsum used in ASKENASY'S experiment we employ a PFEFFER'S cell (p. 13), it would appear probable that higher values will be obtained, values which far exceed those of atmospheric pressure. In this form the apparatus would present a greater likeness to the conditions obtaining in the plant, for the PFEFFER'S cell would correspond to a parenchyma cell of the leaf and the glass tube to a vessel. Let us assume that the cell borders directly on an intercellular space on the one side, and that the vessel, on which the other side abuts, is filled with water and has its lower end plunged into mercury, and that its wall, as in the case of the glass tube, is impermeable to air. When the cell-sap becomes concentrated owing to transpiration having commenced, the water is withdrawn from the vessel and the mercury rises to take its place. The question then is, how high will it rise ? Let us imagine, for instance, that the mercury rises to a height of 150 cm., then there is no question but that it must be supported by the cell ; but one must not imagine that the cell is not affected by it. It must exert a suction on the cell, just as if we had placed an osmotically active solution near one side of the cell. If the height of the mercury still goes on increasing there comes at last a time when this suction becomes equal to the osmotic pressure in the interior of the cell. When this point is exceeded the mercury acts just like a plasmolysing solution. At the same moment any further absorption of water on the part of the cell comes to an end, the water passes backwards into the vessel, and the ascent of the mercury ceases.

Such considerations as these compel us to conclude that continuous water columns in the vessels appear unequal to the task of raising water easily, since they are unable to overcome the great cohesion existing between the particles of water. In proportion as the columns become longer the absorption of water by the leaf-cells becomes more difficult, and must, in the long run, become quite impossible. We are further, for many reasons, unable to calculate how long the water columns may actually be. In the first place, we are ignorant as to whether very lofty trees may not perhaps possess an especially high osmotic pressure in their leaves (and in this relation it must be remembered that DIXON'S (1896) determinations of osmotic pressures in the leaf are by no means above criticism), and in the second place, the power of suction possessed by the water column depends not only on its length but also on the friction which it meets with, owing to the tendency to collapse on the part of the walls of the vessels, as well as on the opposition to its passage through the living cells of the root.

Should we desire to conduct a research with the view of finding out the amount of frictional opposition the water suffers (whether in ascent or descent) in the wood of a tree, we must make it our business first of all to obtain a clear conception of the intimate structure of the vessel, and, above all, to note in what respects it differs from the glass tube which has hitherto served as our model ; i.e. we have to study its length and breadth and the nature of its wall.

The vessel varies greatly in length according to its mode of development. Two extreme conditions may be noted and designated respectively tracheae and tracheids. The term ' vessel ' is generally taken as the equivalent of ' trachea '. We shall follow in this work the suggestions of ROTHERT (1899) in regard to nomenclature. The short and comprehensive title ' vessel ', to include both tracheae and tracheids, is, we think, the more necessary, for both morphological and physiological reasons, since very often, in individual cases, it is by no means certain whether tracheae or tracheids are concerned in the process.

Tracheids are nothing more nor less than much elongated cells which remain entirely closed ; *tracheae*, on the other hand, are cell chains which run through the plant in definite directions, and whose lumina have become united

into one long cavity by absorption of the transverse partitions. In general, tracheids are shorter and narrower than tracheae. The following table furnishes us with data with regard to the dimensions of certain vessels :—

	Width in mm.	Length.
Tracheids of Dicotyledons	—	0·16–1 mm.
,, *Pinus*	up to 0·03	4 mm.
,, *Musa* and *Canna*	0·1	10 mm.
,, *Nelumbium*	0·6	120 mm.
Tracheae of *Mucuna* sp.	0·6	—
,, *Calamus draco*	0·562	—
,, *Wistaria sinensis*	0·2	up to 3 m.
,, *Aristolochia*	0·14	3 m. or more
,, Oak	0·2–0·3	2 m. or more
,, Ash	0·14	—
,, Beech	0·028	—
,, *Ficus*	—	10–66 cm.

When we remember that the water lost by the branch in the process of transpiration streams along the vessels, much in the same way as it does in the glass tube which forms part of ASKENASY'S apparatus, it will be at once apparent to us that the tracheae are much better fitted for water transport than the tracheids. In the first place, they are *wider* than the tracheids, and a well-known law in physics tells us that the amounts of fluid which pass through two tubes under the same pressure are proportional to the fourth power of the radius. In the second place, the vessels are only rarely interrupted by transverse walls, each transverse partition acting as an obstacle to the movement of water. If the resistance to water conduction in the transporting conduits be *alone* considered, it is obvious that the longest and widest vessels would be the best fitted for the purpose. Since, however, we find that in the majority of plants narrow and short tracheids occur in addition to long and broad tracheae, we are compelled to conclude that this difference in the form of the conducting elements corresponds to a difference in function ; wherein the division of labour between the two elements consists, however, it is impossible to say. It is known only that, to a certain extent, the tracheids may take the place of the tracheae, a conclusion which may be arrived at from a knowledge of the fact that the wood of many trees (e. g. Coniferae, *Drimys*, *Trochodendron*, &c.) consists entirely of tracheids, and this may be experimentally determined from deep incisions made right into the pith. The numerous experiments on this subject were first correctly interpreted by STRASBURGER (1891). If several incisions, at suitable distances apart, laterally and vertically, be made in wood which contains *only* tracheae (e. g. *Ficus*), the wood is rendered incapable of performing its functions, because all the conducting channels are interrupted. When tracheids occur as well as tracheae, or when tracheids only are present, the incisions do less damage, because, where the interruptions occur, a *lateral* conduction through the tracheids is effected. This is especially evident if solutions of colouring matters be used ; the *tortuous* path taken by the water in its ascent is shown by the coloured fluid, while normally it may be demonstrated that the water follows a *straight* course by means of the same method.

In addition to a vertical ascent a lateral passage of water in the wood is also possible. Obviously in the case of lateral movement a far greater number of walls must be passed through than in the case of longitudinal movement, whether these be the walls of tracheae or tracheids. But, as we have stated above, the walls present a certain amount of resistance to the movement. Before going more closely into this matter we must study the structure of the wall of the vessel ; a subject which is of interest from other points of view also.

The first thing noticeable with regard to the wall is its peculiar sculpturing. There are no vessels whose walls are *uniformly* thickened for any great dis-

tance ; *thick* and *thin* regions always alternate. The thickenings are annular, spiral, or reticulate. In the spiral and annular vessels the thin portions are of similar form to the thick parts and alternate regularly with them ; in the reticulate type, however, the thin places are circular or elliptical and known by the name of ' pits '. It may be logical, but scarcely customary, to call the thin places in the first two cases ' pits ' also, all the more as there is no sharp line of demarcation between the three types. This is not the place to enter into a discussion of their other peculiarities, though an appearance of very general occurrence may be noted, viz. the attachment of the thickened regions to the original unthickened wall by a somewhat narrower base (Fig. 17), so that a transverse section of the thickening presents a ⊢-shaped appearance. This structure in ' annular ' and ' spiral ' vessels was for long overlooked, and was first demonstrated by ROTHERT's (1899) elaborate researches.

One form of *reticulate* thickening, on the other hand, has been often investigated and known by the name of ' *bordered pits* '. Looked at on surface view (Fig. 18, *2*) the circular or elliptical mouth of the pit appears surrounded by two lines enclosing a ring-like area. It is easily understood that the cavity is due to the deposit of thickening material on the outer rim of the pit, the widening of which brings about the formation of the cavity ; pit canals of

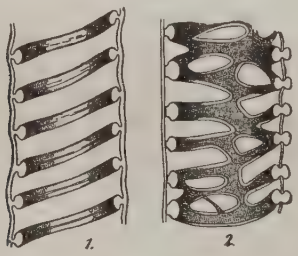


Fig. 17. 1. Spiral vessel of *Cucurbita* in longitudinal section. (After ROTHERT, 1899, pl. 6, fig. 11. × 400.) 2. Reticulate vessel of *Opuntia*. (After ROTHERT, 1899, pl. 6, fig. 30. × 400.)

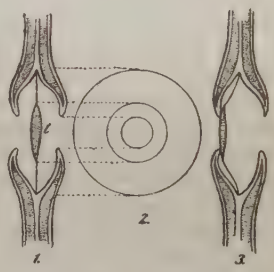


Fig. 18. *1*. Bordered pit of *Pinus sylvestris* in tangential section ; *t*, the torus. *2*. Surface view of the pit ; corresponding regions are united by dotted lines. *3*. As in *1*, but the torus is pressed against the pit on the left. (After RUSSOW, diagrammatic.)

equal width throughout have no such cavity. Bordered pits occur especially frequently on walls which are common to two vessels and are then (as in Fig. 18) bilaterally symmetrical. The median closing membrane possesses the further special peculiarity of having in the middle a lens-shaped swelling (torus) sharply deliminated from the very thin remainder. The closing membrane is not always found medianly situated, as is shown in Fig. 18, *1* ; it is capable of moving in the pit cavity, and, in extreme cases, may be pressed up against one or other of the exits of the cavity (Fig. 18, *3*).

The structure of the bordered pits, as well as the wall of the vessel in general, as is easily seen, must be conducive to the transference of water into a vessel from any element in the vicinity (parenchyma cell or vessel). The walls of a vessel, saturated with water, like those of an ordinary cell, are swollen, and they also allow water to pass through them, although they obviously present a certain amount of resistance to the passage, a resistance which must, *caeteris paribus*, be all the greater the thicker the wall. The closing membranes of the bordered pits will also easily permit the passage of water, but the thickened regions will present resistance. Experimental research amply confirms this *a priori* thesis. Such researches are most conveniently carried out on Coniferae, because in these plants the wood consists entirely of tracheids arranged with great regularity.

The tracheids are on an average about 1 mm. long, 0·02 mm. broad, and as much in depth. In transverse section they are nearly rectangular and so arranged that their walls face radially and tangentially. Bordered pits occur almost exclusively on the radial walls and the tapering ends, the tangential walls being almost entirely free from them. If water be forced through coniferous wood in a *longitudinal* direction it will meet with transverse walls which are about a millimetre apart, but whose resistance to the flow is reduced by the presence of pits in them. If water be forced through in a *tangential* direction it will meet with fifty times as many walls as it will in the longitudinal course; finally, if it be forced through in a *radial* direction it will have to traverse about the same number of walls as in the tangential course, but it will meet with no pits in its journey. After this preliminary statement let us turn to STRASBURGER'S experiments (1891) with the fresh wood of the silver fir, which may be summarized as follows :—

1. A column of water, 50 cm. in height, filters completely through a piece of wood, 8 cm. long, in a *longitudinal* direction in *one* hour: it encounters no opposition worth mentioning.

2. A similar column of water passes in a *tangential* direction through a piece of wood 1–3·5 cm. in length, at the rate of about 4–10 cm. in *20* hours.

3. The opposition is so great in the *radial* direction that passage of the water cannot as a rule be noticed at all, and if the pressure be increased by means of a column of mercury, it is found that 50 cm. of mercury will drive water radially through a piece of wood 1·5 cm. long at the rate of only about 4 cm. in 24 hours, and about 6 cm. in 48 hours.

Similar results had been previously obtained by SACHS (1879, 297) and ELFVING (1882). Thus we are able to understand clearly the significance of the closing membranes of the pits, and may interpret the arrangement in this way, that the margin of the membrane, when in a neutral position, behaves like an ordinary closing membrane, while, when the torus is pressed to one side against the pit opening, this thickened portion of the membrane, to a certain extent, counteracts the activity of the pit. If, therefore, the torus becomes sucked against the opening, owing to extensive abstraction of water and the negative pressure of the air in the vessel resulting therefrom, then any further withdrawal from this vessel must come to an end until this negative pressure becomes general in the vicinity, and the closing membrane of the pit again takes up a neutral position. This explanation, advanced by STRASBURGER (1891), is the most satisfactory hypothesis of the function of the bordered pit, and hence must be cited here, although it has not been conclusively demonstrated (compare SCHWENDENER, 1892, Ges. Abh. 1, 288).

As this torus-formation is not found in the pits of all vessels the ⊢-like thickening of the wall must have a special significance. That this structure has a meaning may be deduced from its very general occurrence; but before we go into this question we must examine more closely into the purpose of the *thickening* itself. Since the thin regions of the wall of the vessel are especially adapted for the passage of water, why is the whole wall not of this uniform thickness? We can scarcely go wrong if we recognize the advantages which the plant gains by the thickening from a *mechanical* point of view. Ordinary parenchymatous cells with thin walls are able to acquire very considerable rigidity by means of osmotic pressure, but the water in the lumina of the vessel remains either at atmospheric pressure or even less; only rarely is the pressure higher than that of the air. Further, if turgid cells surround a vessel, they would be able to press the walls together and so obliterate the lumen, were they not prevented from doing so by the great rigidity of the membrane of the vessel. The membrane acquires this rigidity by means of the thickening as well as in consequence of other physical characteristics, and

the alternation of thick and thin regions may be interpreted as a compromise whereby the wall permits water to pass through whilst at the same time maintaining its rigidity; compromises such as this are frequently met with in the architecture of the plant. From this point of view the narrow connecting bands of thickening deposit are quite comprehensible; they render the existence of broader thin regions possible and, at the same time, do not materially impair the rigidity of the vessel (SCHWENDENER, 1882 and 1892; ROTHERT, 1899). We need not enter into a discussion here of the physiological significance of the individual forms of thickening.

Having now appreciated the relation existing between the structure of the wall of the vessel and the function which the vessel itself fulfils, the next question before us is the explanation of the chemical characteristics of the wall. It is well known that the walls of all vessels are lignified, that is to say, their original cellulose reaction is greatly altered by the infiltration of aromatic substances (hadromal, CZAPEK, 1899). But our knowledge of the subject is very limited; we do not know how the physical characters of the cell-wall are altered, nor whether the vessels are rendered fitter for the performance of their functions by lignification. That lignification is by no means necessary is shown by the fact that it takes place not only in vessels, but also in other elements which have nothing to do with the transport of water. Nor in the present condition of our knowledge are we able to form a judgement on the interpretation to be given to the fact that the closing membranes of the bordered pits are different in their chemical nature from the rest of the wall.

Tracheae have received their name from the organs in animals known by that title, because not only do they possess to a certain extent a similar structure, but also because they were supposed to have a similar function. For long it was believed that they were the *respiratory organs* of the plant and carried air in their interior. That this was an error was shown especially by the researches of VON HÖHNEL (1879) and BÖHM (1879), and since that time it has been conclusively shown that the lumen of the vessel *always* contains water. At certain times, e. g. during the growth of the vessels, and in trees during early spring, when root-pressure is vigorous, the vessels are often completely filled with water; but as soon as transpiration sets in, air is plentifully present in them. Whence comes this air? There are two possible means of entrance. It could enter the vessel as air dissolved in the water already in the root, or it could in the first instance diffuse through its wall in higher regions of the plant (CLAUSSEN, 1901). In both cases the air would remain at first dissolved in the water which fills the vessel. When, however, at the commencement of transpiration, the leaf-cells demand more water from the vessel than it can supply, a vacuum must tend to be formed in the vessel, and into that the air escapes from solution in the gaseous state. Such air-bubbles will have a lower pressure than one atmosphere, and will therefore act in a sucking manner; water will be withdrawn from the neighbouring tracheid, and in it, in turn, air-bubbles under low pressure will appear. This negative pressure of the air in the vessels has been fully demonstrated by v. HÖHNEL. He cut off branches under mercury from actively transpiring trees and herbs, and observed how the metal was forced far up into the lumina of the vessels by the external atmospheric pressure, overcoming the very obvious capillary depression. The rarefaction of the air reaches its maximum during the greatest transpiration; but it may disappear again entirely during the night owing to continuous entrance of water through the root, whilst the vessels, when the air becomes dissolved once more, become full of water. If the rarefaction of the air continues for a longer time, air from *outside* enters through the walls of the vessels. When the air-pressure within and without the vessel is by this means equalized, the vessels cannot be again completely filled with water, and further entrance of air, in the long run, will inter-

fere with the capacity of the wood for transporting water. We shall refer later on to the conditions which bring about a diminution in the air in the vessel.

Before considering in greater detail the distribution of air and water in the vessels, we must briefly allude to one consequence of the reduced pressure of the air in the vessels which is familiar to every one in the course of everyday life, and which is also of great importance in physiological experiments. If a branch be cut off, without taking any special precautions, and placed in water, it very soon begins to wither, because, during the operation, air has entered the open vessels and may ascend to a variable height in accordance with the amount of negative pressure in the vessels. If, however, the branch be cut off under water, the water is forced into the vessels by atmospheric pressure, and the cutting does not wither. The entrance of water naturally equalizes the negative pressure, but this may reappear, if the cut surface of the twig becomes impermeable to water owing to slime exuded from the plant accumulating on it, or owing to the varied methods of blocking up of the vessels due to the plant's own activity (WIELER, 1888), or, finally, owing to the action of Bacteria. Continued transpiration again induces withering, but this we can easily overcome by forming a freshly cut surface above the first made, of course also under water.

The negative pressure of the air in the vessels, as we have already seen, must render the determination of the distribution of water and air in the vessel very difficult. On undertaking an investigation on selected branches, as a rule it will be found that the results arrived at are perfectly useless, because, in the process of cutting, the basal parts of the branches have become filled with air. It is preferable, therefore, to cut the branch off at two places at the same time, or to isolate a cylinder from a tree trunk with a Pressler's growth borer: by either means the air, oil, or mercury enters both sides at the same time, and hence the air-bubbles originally present, mixed up with water, are forced into the central region of the preparation, where not only the length of the parts with water and air may be measured, but also where the initial rarefaction of air may be easily determined. The rarefaction of the air is, according to SCHWENDENER (1886), generally only about one-third of an atmosphere, rarely values of a quarter to one-fifth of an atmosphere are reached, almost never less. Whilst only one air-bubble can occur under such conditions in the middle of a tracheid, driven there by the pressure of water from both ends, numerous air-bubbles always occur in the tracheae, separated by water columns. The alternating air-bubbles and water columns show not inconsiderable differences in size, still the following *averages*, which SCHWENDENER (1886) obtained in case of *Fagus sylvatica*, may stand for a fair representation of the usual distribution of water and air in the vessel :—

	Stem May 30.	Stem May 31.	Branch 2 years old May 31.	Branch 10 years old June 2.	Twig 2 cm. thick June 23.
Air-bubbles (mm.)	0·364	0·294	0·220	0·322	0·378
Water column (mm.)	0·182	0·182	0·160	0·091	0·108
Total (mm.) . .	0·546	0·476	0·380	0·413	0·486

On an average a segment of the chain composed of air-bubbles and drops of water is about 0·5 mm. in length. Such a series is known as a JAMIN'S chain, and it is obvious that the movement of water in such a chain must take place under conditions essentially different from those obtaining in a tube completely filled with water.

SCHWENDENER does not say from what part of the wood the vessels upon which he made his observations were taken, and, according to STRASBURGER'S (1891) results, the amount of air in the vessels in the periphery of the wood, as also in the younger parts generally, is much less than that in the older annual

rings. Indeed, in many plants the youngest annual ring normally exhibits long, continuous water columns entirely destitute of air-bubbles, while, further in, the wood contains so much air that its conducting power is completely destroyed.

The physical apparatus—a transpiring osmotic cell with a glass tube attached filled with water—which we used as a model of what is found in a tree, differs in many essentials from the reality. The glass tube has a wall which is impermeable to water and air, it forms a continuous channel, and is also full of water throughout. The vessel in the plant is permeable both to water and air, it has always here and there transverse walls which interpose a certain amount of resistance to the movements of water, and, furthermore, it is generally not continuously filled with water but with a chain of alternating bubbles of water and air. Now if, as DIXON and ASKENASY affirm to be the case, the cohesion of water particles plays an important part in the ascent of sap, the vessels ought to contain no such chains. It cannot be denied that, as NÄGELI (1866), DIXON and JOLY (1895), and ASKENASY (1895, 16) have shown, the entrance of air-bubbles into the vessel may be prevented by certain factors, but in reality everything goes to show that, as a rule, the entrance of air into a vessel transporting water can no longer be doubted. For this reason alone the cohesion hypothesis must be given up, or, at least, it must be admitted that it can play no very important part in the process. [Compare STEINBRINCK on the cohesion hypothesis (Ber. d. bot. Gesell. 22, 526, 1904).] Further, even though the columns of water in the vessels were continuous, the cohesion hypothesis could not be accepted as proved. What we require in any theory of water movement is proof not only that the water can be raised to a *certain height*, but also that it can rise to this level in *sufficient quantity*.

The knowledge which we have previously acquired on the subject of the ascent of water as a result of capillarity is instructive. It is well known that the concave meniscus, which forms in a glass tube immersed in water, has less surface tension than a corresponding level surface of water, and that, in consequence, water rises in a capillary tube above the level of the surrounding fluid. The height to which the water ascends depends upon the concavity of the meniscus, and that, in turn, on the diameter of the tube, and it is easy to see that, given a sufficiently narrow tube, any height may be attained. If we consider the cavities of the vessels filled with water as capillary tubes in a microscopic sense—and not much can be urged against this assumption—then it is conceivable that very tall trees might be supplied with water by capillary attraction. NÄGELI (1866) and STRASBURGER (1891) have, however, shown that a capillary ascent of this kind is quite insufficient to replace the water lost in transpiration. Although the principle under discussion is, from a purely physical point of view, perfectly correct, it does not come into play under the conditions existing in the plant. The same might be affirmed of the cohesion hypothesis.

Taking into account all the facts which have been observed, we may formulate our problem in this way: How can the ascent of sap be effected in the plant if chains of water and air-bubbles (JAMIN'S chains) occur in the vessels and if transpiration is always effecting a suction at their upper ends? On this question we have to thank, more recently, SCHWENDENER (1893) and STEINBRINCK (1894) for valuable information. It is possible that the ascent of water in a JAMIN'S chain may take place in one of two ways, either the whole chain, or at least its upper segments (including both air-bubbles and water drops), moves upwards, or the water alone moves while the air-bubbles are stationary. Let us look first at the movement of the *whole* chain. Let us imagine a long vessel or a glass tube filled with air and water segments, each 1 mm. in length, with a suction pump acting at the upper end; by this means the air-bubbles will be stretched out and the water columns will be pulled upwards. It is also obvious that the topmost air-bubble will be extended most, and, in the long run, will show a pres-

sure corresponding to the tension of water vapour. On the next air-bubble, lying farther down, there will be the pressure of a water column of 1 mm. in height, and hence it will not be stretched quite so much as the top one and so on, each more deeply placed air-bubble being less stretched than the one next above it. But there is another consideration to be taken into account. In a horizontally placed tube, where the effect of the weight of the water columns is eliminated, each separate segment of the chain opposes the movement in a way not quite understood theoretically, but admitting of practical estimation. SCHWENDENER found it to be approximately the weight of 4 mm. of water. On the whole each small column of water opposes the upward ascent with a force equal to the weight of 5 mm. of water. Under these conditions, and bearing in mind that in the uppermost bubbles the tension is never that of water vapour, but very much higher (one-third to one-fifth of an atmosphere), SCHWENDENER estimated that the suctional power of the leaf was transmitted backwards not quite 5 m. At this distance an air-bubble would exhibit only atmospheric pressure.

The assumed length of the air-bubbles, viz. 1 mm., is, as a matter of fact, too great ; but if one imagines them smaller, then their suctional efficacy is all the less. On the other hand, the amount of the opposition to the movement of the separate water columns has perhaps been placed too high, and thus the suction would stretch them out more. On the whole, however, SCHWENDENER'S criticism may be accepted as correct ; the suction of the air-bubbles which raises the water can act only a few metres backwards from the top of the tree, perhaps to the base of the branches, but certainly not into the stem. Other forces must, therefore, be forthcoming to effect the elevation of water in the stem. Again, negative air pressure in the vessels, which can exist independently of transpiration (compare p. 76), occurs in the trunk of a transpiring tree, and it has never yet been observed that this pressure increases continuously from the apices of the branches towards the base of the stem. We feel bound to conclude from this that there is something in SCHWENDENER'S assumption not in accordance with fact.

There are other difficulties which stand in the way of our acceptance of the idea of movement of the entire water and air chain. This theory assumes that these vessels are continuous throughout the entire tree, while in reality the vessels have always only a limited length. Furthermore it would be a puzzle to determine where the air goes to when the water is absorbed by the cells at the upper ends of the vessels. It is obvious that it would result in the rapid formation of a cavity filled with air at low pressure, whose removal later would be impossible. Under these conditions it will repay us to keep the other possibility before us, and to inquire whether it may be possible that a movement of the water in the chain takes place, though the air-bubbles remain stationary. Such a hypothesis as this does not appear unjustified, because in the tracheids, e. g. of coniferous wood, the conditions completely forbid a movement of the air-bubbles for any great distance.

How then can suction, exerted on the upper end of the water column in a long vessel, be transmitted downwards while the air-bubbles in the vessel remain stationary ? Three ways are possible ; either the water flows between the air-bubbles and the wall, or in the wall itself, or, finally, through the neighbouring cells. In this last case, if no cell or vessel in the neighbourhood is capable of interfering in virtue of its vital activity, we may imagine a system of several vessels lying one behind the other and acting together, replaced by a *sinuous* vessel subdivided frequently by transverse walls. This assumption obviously has no advantages over a straight and continuous vessel filled with water : there is nothing to be gained by discussing it further. The difficulties in the way of a movement of water in the wall itself have been already mentioned (p. 48), and these are sufficient to make the second possi-

bility improbable. Only the first method remains, viz. that the water must move between the air-bubbles and the wall of the vessel. This view was first put forward by VESQUE (1883) and subsequently maintained by STRASBURGER (1891). It must first of all be noted in this connexion that owing to the sectional outline, which is far from being a circle, and owing to the frequent occurrence of local thickenings on the wall—especially if these be of the spiral variety—a close adhesion of the air-bubbles to the wall of the vessel is prevented, and quite large spaces may exist between the bubble and the wall, in which a movement of water would appear possible, and this would still be the case if only the so-called adhesive water film between the air-bubble and the wall were present. VESQUE has, as a matter of fact, seen a stream of water flowing past the stationary air-bubbles, both in plant vessels and in capillary glass tubes, and COPELAND has more recently (1902) confirmed VESQUE'S observations. Although there can be scarcely any doubt of this fact still it is not possible at present to explain physically the ascent of sap in this way. By this arrangement we have continuous columns of water which, every here and there—at each air-bubble—have a diminished diameter ; this periodic lessening of the diameter decreases the tendency to sink on the part of the water column, on account of the additional friction, and must, for the same reason, hinder its ascent. Sinking of the column appears to be inevitable as soon as its weight exceeds the resistance to filtration offered by the root-cells and the friction in the vessels. If, however, these resistances are sufficiently great to prevent this *sinking*, how a force arises which is capable of effecting a *lifting* of the water is not apparent. One cannot resist the impression that the more intimate *physical* conditions of the ascent of sap in the plant have not as yet been clearly made out, and we must still wait for further elucidation of these phenomena by experimenting with apparatus more nearly resembling vessels than the glass tubes which we have hitherto employed to represent the vessels. An apparatus of this sort has been devised by COPELAND (1902). He has shown that if a tube, over 12 m. high, be filled with plaster of Paris, water, and air, evaporation at the upper end is followed by an ascent of the water. He has observed, further, that the pull of transpiration is active much further down than SCHWENDENER thought, but he is unable to explain physically the way in which his own apparatus works. It seems to us most important to elucidate, first of all, the distribution of negative pressure in the tree. According to STRASBURGER'S (1891) observations, and also according to those of PAPPENHEIM (1892), the negative tension at the tips of the branches does not appear to differ from that at the base, and a negative pressure apparently exists at the bottom of the tree, as is proved by the sucking up of water through the cut surface. Whether this is dependent on the suction exerted by the leaves, or arises from other causes, it should not be difficult to determine. If it be due, as appears to us likely, to the activity of the leaves, then the osmotic suction must certainly reach down to the root (in opposition to what SCHWENDENER found), and then one might truly say that *transpiration provides the force which causes the ascent of sap*. But even then physical research would require to show how it was that the air in the vessels at the base of the stem was not compressed by the superincumbent water columns.

In addition to *purely physical forces* the activity of the *living cells* is frequently brought forward as a cause of the ascent of sap. In a certain sense their co-operation cannot be doubted, in so far, that is to say, as the living cells build up the vascular system and develop into forms suitable for performing this function in the plant. The vessels, when they begin to act as water conduits are already filled with water. If the water be withdrawn from the vessels they cannot be again filled by the plant's agency, their capacity is for ever lost, and the plant dies unless water be injected artificially into the vessels.

According to R. HARTIG (1883, p. 73) the spruce stem becomes incapable of conveying water when the lumina of the tracheids are still more than half full of water. Apart from this, however, living tissues, especially the parenchymatous cells, which are almost always associated with the vessels, may take part in water conduction directly or indirectly. WESTERMAIER (1884), GODLEWSKI (1884), and JANSE (1887) have assumed that the parenchyma play a direct part in the process. The essential point in all these theories is that parenchymatous cells abstract water from one vessel and hand it on to one higher up. A complete discussion and criticism of these views (ZIMMERMANN, 1885, SCHWENDENER, 1886) need not be presented here, since, owing to the researches of STRASBURGER, all such vital theories have received a severe blow, if indeed they have not been directly disproved. Further, no positive evidence has been advanced in support of these theories, and one accepted them because purely physical explanations appeared to be inadequate.

Experiments on the ascent of sap in dead branches had been previously carried out, but for the most part these were confined to killing short lengths and establishing the fact that they were permeable to water. Naturally, it cannot be concluded from such experiments whether, in *stems of any length*, water conduction goes on after the death of the parenchyma. Such researches on a large scale we owe to STRASBURGER (1891, 1893). He killed long branches of *Glycine*, by placing the lower leafless part, which was 10–12 m. in length, in boiling water, and observed that an eosin solution rose in them from the cut end to a height of 16·8 m.; still the leaves which were present at the uninjured end of the stem remained alive for only a few days, after which they dried up and fell off. In all probability the deficiency in the supply of water to the apex was not due to the death of the parenchyma but to the fact that masses of slime and other obstructions entered the vessels, in the way we have already described.

STRASBURGER has also killed long reaches of plant stem otherwise, e. g. by poisons, and proved that they are still capable of conducting water in the dead condition. The following extract may be selected from the description of one of his numerous experiments (STRASBURGER, 1893, p. 10).

'The summer oak selected for experiment was 21·7 m. high, 27 cm. thick, at a height of 10 cm. above ground, and 75 years old. On June 28, about 4 p.m., the tree was sawn off, obliquely, 10 cm. above ground, while water flowed rapidly in the cut. The severed trunk was at once raised to a vertical position and suspended in a tub of water. It remained in this tub about half an hour, whilst its cut surface was cleaned and smoothed with a sharp knife. The tree was then put into a vessel filled with a saturated solution of picric acid, which is intensely poisonous to the plant. It was sunk about 20 cm. in this fluid.' The upper limit of the solution in the vessel was noted and the amount kept constant by filling the vessel up to this level morning and evening each day.

The amounts of fluid absorbed by the stem were as follows :—

	I.		II.		III.		IV.		V.		VI.		VII.		VIII.		IX.	
	p.m.	N.	D.	N.	D.	N.	D.	N.	D.	N.	D.	N.	D.	N.	D.	N.	D.	N.
Hours . .	4	14	9	15	9	15	8	14	13	11	12	12	12	12	12	12	12	12
Litres . .	5	6·5	6	5·5	5·75	4·2	6	4·7	5·8	4	3·7	3	2·4	2·4	1·45	0·7	0·3	1

It is clearly seen that considerably greater amounts of water were taken up at first during the *day* than during the *night*, although the period of observation termed 'night' for short was markedly longer than the 'day'. Later, after the leaves had died, this periodicity ceased and the absorption of fluid as a whole fell off very considerably. After the fourth day the picric acid had risen up the stem to a height of 15 m. and had killed these parts; when fuchsin was added to the solution the rise of this colouring matter could be followed in the *dead* stem. At the end of the experiment, on the tenth day, it was

shown without doubt that fuchsin had been carried to the top of the tree without the co-operation of living cells, because parts of the stem investigated as high as 21·8 m. from the ground were tinged with the pigment.

The experiment just described is undoubtedly among the most interesting of those which have been carried out on the subject of water conduction, but it cannot be regarded as absolutely convincing, for it raises many doubtful issues. First of all there is the question why the absorption of water decreases so rapidly. Probably because evaporation from the leaves ceases, as a consequence of withering; but we do not know whether the withering of the leaves is due to death by the poison or is a result of reduced water supply. It is quite possible to assume that water still rises in the dead stem, but no longer with the requisite rapidity, or in amount equal to what the transpiring leaves demand. A completely convincing experiment must show that the leaves which are supplied with water by a dead stem are able to remain for a long time alive. [URSPRUNG (Beihf. z. bot. Centrbl. 1904, 18, 147) has advanced certain important criticisms tending to disprove STRASBURGER'S experiments, but his own experiments do not convince us that living cells conduce to the ascent of sap. Compare DIXON, 1905 (Proc. R. Dublin S., 11, No. 2); URSPRUNG, 1905 (Bot. Ztg. 63, 11 Abt. 241), and JOST, 1905 (Bot. Ztg. 63, II Abt. 243).]

When we spoke above of an indirect action of the living cells on the conduction of water, we meant an action not dependent on a sucking and pumping upwards of air, but referred, rather, to the influence of living cells on the air in the vessel. NOLL (1897), in a preliminary treatise, has noted the fact that gases which are injected into the vascular system undergo changes, and DEVAUX (1902) has observed a negative pressure in the air of the vessel on the *stoppage of transpiration.* This he attributes to the withdrawal of oxygen from the vessels owing to the respiration taking place in the living cells. Further investigations have now to show whether negative pressures in larger areas are really produced in the wood by this means. If that be the case, then it would be impossible to doubt the suctional action of the expanded air-bubbles, and the living cells would then play an important part in the ascent of water.

The summary we have given above shows that our knowledge up to the present of the causes of the ascent of water is in many respects very imperfect. A complete exposition of the voluminous literature (COPELAND, 1902) is out of the question here; it would lead us to no definite results, for we are ignorant even yet of the answers to the most elementary questions. It may be difficult, perhaps, to clear up *entirely* the darkness which surrounds this question, although we may still look for future researches to throw light on the problem, if these, as we have often already emphasized, pay more attention than hitherto to the *quantity* of the water to be raised as contrasted with the quantity actually raised.

Bibliography to Lecture VI.

ASKENASY. 1895. Verhandl. d. naturhist.-med. Vereins Heidelberg, N. F. 5.
ASKENASY. 1896. Ibid.
BÖHM. 1879. Bot. Ztg. 37, 225.
CLAUSSEN. 1901. Flora, 88, 422.
COPELAND. 1902. Bot. Gaz. 35, 161.
CZAPEK. 1899. Zeitschr. f. phys. Chemie, 17, 143.
DARWIN, VINES, JOLY. 1896. Report of a discussion on the ascent of water. Annals of Bot. 10, 630.
DEVAUX. 1902. Compt. rend. 134, 1366.
DIXON and JOLY. 1895 A. Proc. Roy. Soc. 57, 3.
DIXON and JOLY. 1895 B. Phil. Trans. B. 186, 563.
DIXON. 1896. Proc. R. Irish Acad. 4, 61.
ELFVING. 1882. Bot. Ztg. 40, 713.

GODLEWSKI. 1884. Jahrb. f. wiss. Bot. 15, 602.
HALES. 1748. Statik der Gewächse. Halle.
HARTIG, R. 1882. Unters. aus d. forstbot. Institt. München, 2, 1.
HARTIG, R. 1883. Ibid. 3, 73.
HARTIG, Th. 1861. Bot. Ztg. 19, 22.
HOFMEISTER. 1862. Flora, 45, 97.
HÖHNEL. 1879. Jahrb. f. wiss. Bot. 12, 47.
JANSE. 1887. Jahrb. f. wiss. Bot. 18, 1.
MCNAB. 1871. Trans. Bot. Soc. Edinburgh, 11, 45
NÄGELI. 1866. Sitzungsber. Bayer. Akad. d. wiss. Bot., Mitt. 2, 369 and 429.
NERNST. 1900. Theoret. Chemie, 3rd ed., p. 165.
NOLL. 1897. Sitzungsber. Niederrhein. Gesell., November.
PAPPENHEIM. 1892. Bot. Centralb. 49, 1.
PFEFFER. 1892. Stud. z. Energetik. (Abh. Kgl. Gesell. d. Wiss. Leipzig, 18.)
REINGANUM. 1896. Annalen d. Physik (Wiedemann), N. F. 59, 764.
PFITZER. 1877. Jahrb. wiss. Botan. 11, 177.
ROTHERT. 1899. Bullet. de l'Acad. de Cracovie, 34.
SACHS. 1873. Arb. Würzburger bot. Inst. 1, 288.
SACHS. 1878. Ibid., 2, 148.
SACHS. 1879. Ibid., 2, 291.
SCHWENDENER. 1882. Die Schutzscheiden. Abh. d. Berl. Akad. (Ges. Abh. 2).
SCHWENDENER. 1886. Sitzungsber. Berl. Akad. 561 (Ges. Abh. 1, 207).
SCHWENDENER. 1892. Ibid. 911 (Ges. Abh. 1, 256).
SCHWENDENER. 1893. Ibid. 835 (Ges. Abh. 1, 298).
STEINBRINCK. 1894. Ber. d. bot. Gesell. 12, 120.
STRASBURGER. 1891. Bau und Verrichtungen der Leitungsbahnen (Histol. Beitr. 3). Jena.
STRASBURGER. 1893. Ueber das Saftsteigen (Histol. Beitr. 5). Jena.
VESQUE. 1883. Ann. sc. nat. 15, 5
WESTERMAIER. 1884. Sitzungsber. Berl. Akad. 1105.
WIELER. 1888. Jahrb. f. wiss. Bot. 19, 82.
WIELER. 1893. Cohn's Beitr. z. Biologie, 6, 1.
ZIMMERMANN. 1885. Berichte d. bot. Gesell. 3, 290.

[Many of the questions with which this lecture deals have been discussed by EWART, 1905 (Phil. Trans. B. 198, 41–85; abstracted in Proc. R. S. 74; compare Ann. Bot. 443). Into the results of his studies it is unfortunately impossible to go.]

LECTURE VII

ASH. I

ALL plants contain greater or less quantities of incombustible substances, and small fragments of the cell-wall or starch granules leave behind them, on combustion, demonstrable quantities of ash. The experiences of everyday life confirm this. Every one in days gone by had an opportunity of seeing wood-ash, but, perhaps, this opportunity does not occur quite so frequently in these days of coal consumption; still cigars are smoked everywhere, and they illustrate the relatively large proportion of ash present in plant organs. No modern investigator has any doubt that all these mineral constituents of the plant must be obtained from without, and in the main from the soil. It is, therefore, most instructive to note that in earlier times this, to us, self-evident fact was thought to require definite proof, and that, even *after* the establishment of the law of the indestructibility of matter, famous academies suggested prize essays with the object of determining whether this law held also for *organic* nature. For instance, in 1800, the Berlin Academy formulated this question :—

'By what means are the earthy constituents obtained which, as a result of chemical analysis, are found to be in the various indigenous cereals? Do they enter these plants in the same form as they are found in them, or are

they produced by the agency of the vegetative organs themselves ?' The answer given by SCHRADER (1800) to this question ran as follows :—' Plants develop these ash constituents by their own vital force.' Almost forty years later (1838), the Academy of Göttingen again formulated almost identically the same question :—' Whether the so-called inorganic elements which are found in the ash of plants are still to be found there when they are not supplied to the plant from without ?' Such a question was certainly somewhat out of date. DECANDOLLE's Plant Physiology (1831), (German Edition, 1833, I, p. 388), contains a refutation of SCHRADER's view.

The principles of chemistry had now, however, been established on a wider basis, and the answer given to the question was totally different. WIEGMAN and POLSTORFF (1842), by their researches, clearly proved the correctness of the view now held.

Closer inquiry into the conditions governing absorption shows us clearly that these substances pass through the external walls of plants in a state of *solution*, since these walls are impermeable to solids. Not only the medium of solution, water, but also, as a general rule, the materials of the ash are taken up through the cells of the root ; only in rarer cases, e. g. in many epiphytes, do the leaves take part in the absorption. The law of osmosis governs this absorption and determines the nature and amount of the mineral matter absorbed.

The *nature of the salts* depends, in the first instance, on the permeability of the protoplasm, but exhaustive researches are still required to show to what degree protoplasm is permeable to the inorganic salts which in nature come into relation with the plant. So far it has been clearly established that the ash of plants contains by no means all the mineral matters which occur in the soil or in the water. Aluminium, for instance, is widely distributed in nature, and many of its compounds are soluble in water, but, nevertheless, it is absent entirely, or almost entirely, from the majority of plants, although it does occur abundantly in a few species. On the other hand, we know that iodine is present in sea-water only in such small amounts that it can scarcely be detected, yet many Algae take it up in relatively large quantities. Generally speaking, the mineral matters occur in the plant in *proportions* quite different to what they do in the outside world. This is illustrated by WOLFF's (1871, I, 132) analysis of the ash of *Lemna trisulca*, as compared with that of the water in which the plant was grown.

Minerals present in 100 parts of Ash.

	K_2O	Na_2O	CaO	MgO	Fe_2O_3	P_2O_5	SO_3	SiO_2	Cl
Water .	5·15	7·60	45·56	16·00	0·94	3·42	10·79	4·23	7·09
Lemna .	18·29	4·06	21·86	6·60	9·57	11·35	7·91	16·05	5·55

From this summary it is impossible to say whether these substances occur in the *cell-wall* or in the *protoplasm* ; if in the latter we are still ignorant as to the *permeability* of protoplasm to these materials. Because a large amount of iron is taken up it does not follow that the protoplasm is *easily* permeable to it, and, conversely, the fact that lime is present in relatively less amount in the plant than in the water must not be taken as indicating that protoplasm is *less permeable* to that substance. We have already seen, in speaking of osmosis, that, given that protoplasm is permeable to a substance, no *storage* can take place in the plant unless the substance be altered in some way after its absorption. The most obvious example is the coloration which is produced by very dilute solutions of methylene blue. This is only possible if the methylene blue becomes separated out in insoluble combinations. We are, however, ignorant in individual cases wherein the changes in inorganic substances consist which prevent their exosmosis and facilitate their accumulation. From

numberless analyses we learn that such accumulations take place in individual plants specifically differently, and further, that different species may grow in the same situation and yet show quite different ash constituents. As an example we may take the analysis which GRANDEAU and BOUTON (1877) have made of the mistletoe, and of the different media from which it obtains its ash :—

	Percentage of Ash in dry wt.	In 100 parts of pure Ash.								
		K_2O	Na_2O	CaO	MgO Mn_2O_3	Fe_2O_3	P_2O_5	SO_3	SiO_2	Cl
Poplar	3·04	6·56	2·82	66·47	8·20	2·38	4·76	1·49	5·81	1·64
Poplar Mistletoe .	3·46	16·09	2·04	32·55	9·21	5·40	26·29	2·09	4·79	1·47
Robinia	2·06	2·35	0·47	75·04	2·51	1·88	3·45	0·78	11·77	1·72
Robinia Mistletoe	2·13	15·90	2·58	45·39	6·72	2·20	12·02	2·74	6·41	2·01
Fir	1·61	8·40	2·03	67·43	7·12	1·01	7·89	2·80	2·03	1·27
Fir Mistletoe . .	3·14	30·79	trace	27·13	12·19 10·67	1·52	13·11	3·35	1·22	trace

This table clearly shows that the mistletoe has markedly less silicic acid, less lime, and much more potash and phosphoric acid than its host. Moreover, the plants growing on these three different substrata are by no means similar in chemical composition, and the differences between them cannot be in any way referred to corresponding differences in the substrata. These differences are peculiar to the individual and are at present inexplicable. It is self-apparent that a definite species can, *caeteris paribus*, take up more of a substance from a soil which contains it abundantly than from one which contains little, as is shown by MALAGUTI and DUROCHER'S (1858) work on the analysis of lime as a constituent of plant ash.

The total amount of ash in the example given above is very limited and constitutes only a small percentage of the dry weight, but in other cases, the amount is quite considerable. In addition to the large table given below, the first column of which shows this fact prominently, we may extract the following figures from WOLFF'S 'Ash Analysis' (I, 137), dealing with several common weeds grown on similar soils. The ash of *Rumex acetosella* amounted to 8·14 per cent., of *Geranium dissectum* to 9·98 per cent., of *Sedum telephium* to 11·96 per cent., and *Myosotis arvensis* to 17·85 per cent. of the dry weight in each case. Far larger quantities of ash are found in seashore plants ; e. g. 16·51 per cent. in *Aster tripolium*, 17·91 per cent. in *Artemisia maritima*, and 31·57 per cent. in *Chenopodium maritimum*. Although we must attribute the large proportion of ash in these cases to the presence of large amounts of common salt in the sand, still we find other conditions in other cases tending to increase the amount of ash constituents. We need refer here only to transpiration. On grounds which are easily understood, plants which transpire freely are far richer in ash than those which transpire feebly, and the leaves, being the organs of transpiration, appear to contain most. On that account transpiration is of great value to the plant, because, as we shall see presently, the constituents of the ash are essential to its well-being, and by no means superfluous or injurious.

The individual *elements* which occur regularly in the ash of *all* plants are only eleven in number, i. e. :—chlorine, sulphur, phosphorus, silicon, and the metals potassium, sodium, calcium, magnesium, iron, aluminium, and manganese. As a rule the last two occur as traces only, the rest abundantly. The following table (WOLFF, 1880) shows the composition of the ash of certain plants, but only the nine principal elements are noted ; the special abundance of an element is emphasized by italics.

	% of Ash.	In 100 parts of pure Ash.								
		K_2O	Na_2O	CaO	MgO	Fe_2O_3	P_2O_5	SO_3	SiO_2	Cl
1. Tobacco leaves . .	*17·16*	29·09	3·21	36·02	7·36	1·95	4·66	6·07	5·77	6·71
2. Potato tubers . . .	3·79	*60·06*	2·96	2·64	4·93	1·10	16·86	6·52	2·04	3·46
3. Spinach	16·48	16·56	*35·29*	11·88	6·38	3·35	10·25	6·87	4·52	6·20
4. Oak bark (150 years).	7·20	4·36	0·34	*92·82*	1·19	0·29	0·39	0·27	0·55	—
5. Beech in flower . .	6·86	32·29	1·95	*34·91*	10·90	1·08	9·64	3·23	2·69	3·78
6. Almonds (seed) . .	4·90	27·95	0·23	8·81	*17·66*	0·55	43·63	0·37	—	—
7. Italian clover . . .	9·87	33·42	13·01	12·73	6·53	*5·86*	4·63	14·41	4·50	—
8. Wheat (fruit) . . .	2·14	30·51	1·74	2 82	11·96	0·51	*48·94*	1·32	1·46	0·47
9. Horse radish (root) .	8·47	38·96	2·10	10·10	3·66	1·51	10·39	*24·72*	7·20	1·36
10. *Equisetum telmateia* .	*26.75*	8·01	0·63	8·63	1·81	1·42	1·37	2·83	*70·64*	5·59
11. Barley after flowering	6·47	25·44	0·75	5·77	3·03	0·42	10·29	2·94	*49·83*	3·77
12. Celery (root) . . .	11·04	43·19	—	13·11	5·82	1·41	12·83	5·58	3·85	*15·87*

In addition to the problem as to how the constituents of the ash enter the plant we have the further question as to whether they are of value to it or merely accessories accidentally introduced along with the water. SENEBIER (1800) and SAUSSURE (1804) have already shown that certain *minerals* are essential to the plant as *food-stuffs*; this view LIEBIG (1840) supported very strongly, and owing to his authority it received general acceptance, although, strictly speaking, it was not exactly proved till later. The methods employed for this purpose are two in number. Both were intended, at the same time, to show whether *all* or *which* of the ash constituents found in the plant were *essential*. Important experiments for this purpose were first carried out by Prince SALM-HORSTMAR (1856), who employed the first method. He cultivated the plants, following the example set by WIEGMAN and POLSTORFF (1842), in insoluble artificial soils, to which were added the materials which were to be investigated. He used, e. g., soil composed of pulverized rock crystal and carbon obtained from candy sugar, whilst WIEGMAN and POLSTORFF worked with platinum filings and sand. SALM made out that silicon, phosphorus, sulphur, potassium, calcium, magnesium, iron, and manganese were essential to the normal development of oats, but he was doubtful as to the significance of chlorine. Although his results could not be completely substantiated, still they were very valuable in one point, inasmuch as they proved that *sodium*, though never absent from the ash of plants, was not to be included among the essential elements of oats (although SALM believed it to be essential in other cases). This fact is all the more remarkable when one remembers that sodium has important functions to perform in the higher animals.

The other method—the so-called *water-culture* method—is of the greatest value for our purpose. Although SAUSSURE (1804) early in the century grew *Bidens* and *Polygonum* in water, SACHS (1860) and KNOP (1860) were the first to cultivate, experimentally, land plants in such a way that their roots, immersed in a watery solution of various salts, could supply their requirements so far as inorganic salts were concerned, and proved that the plants cultivated in this way showed a large increase in their dry weight. An increase in dry weight, especially a large increase, is a valuable criterion of the success of such a culture, but we should fall into serious error were we to conclude from this that a plant grows only if it be supplied with all the necessary food materials. Growth can take place without increase in dry weight, and indeed without the absorption of water. Again, we should be totally wrong were we to conclude from the fact that plants, without taking up nitrogen, can reach a weight three and a half times that of their seed (BOUSSINGAULT, 1860), that nitrogen was not necessary for growth. From the observations of many investigators it has been shown that maize can develop under the most favourable

conditions from 60 to 370 times, and that buckwheat can increase 1,000 times the weight of its seed; an increase in dry weight of this amount may well be described as abundant. Moreover, seedlings may show an increase in weight without any absorption of ash constituents from the surroundings, and this is explained quite simply by the not inconsiderable quantity of ash which the seeds contain. Beans, for example, may, according to BOUSSINGAULT, develop up to the flowering stage entirely without nutritive salts and, in consequence, double or quadruple their dry weight. We see from such illustrations that it is necessary to eliminate the reserves in the seed entirely, if we desire to prove the essential character of an element which may be present in small amounts in the seed, but which amount may, for the purposes of development, be large enough.

Without going into details we may mention only, with regard to the water-culture method, that it is the custom, as a rule, to start from seeds which have developed their principal roots in sawdust. The seedlings are then fixed in the cork of a vessel of sufficient size, so that the stem is allowed to grow upwards and develop in light and air, while the root branches in the vessel containing the nutritive solution (Fig. 19). It is essential to prevent light from entering the vessel, and this is done most effectively by sinking it in soil and at the same time time keeping it at a uniform and not too high temperature.

The following substances are employed as nutritive salts dissolved in water so as to form a solution of a concentration of a few parts per thousand:

I.		II.		III.	
BIRNER and LUCANUS (1866).		KNOP (1868, 606; 1884).		SACHS (1882, 342).	
Magnesium sulphate, about	0.5 g.	. . 0.25 . .		. . . . .	0.5 g.
Calcium nitrate . .	1.5 g.	. . 1.00		Sodium chloride . .	0.5 g.
Acid potassium phosphate	1.0 g.	. . 0.25		Potassium nitrate .	1.0 g.
Ferric phosphate . .	1.1 g.	—		Calcium sulphate . .	0.5 g.
		Potassium chloride .	0.12 g.	Calcium phosphate	
		Ferric chloride . .	trace	(finely ground) .	0.5 g.
				Ferric chloride . .	trace

The first of these nutritive solutions is the simplest, and with its aid BIRNER and LUCANUS were able to obtain a complete culture experiment with oats, where the increase in dry weight was equivalent to 138 times the weight of the seed. If we ignore for the present the nitrogen, which we will study later, and which does not really belong to the ash constituents at all, we find that the plant requires the following six elements: potassium, calcium, magnesium, sulphur, phosphorus, iron. It can do without the other two elements, silicon and manganese (which SALM-HORSTMAR believed to be essential), and also without chlorine, as to whose indispensableness SALM was doubtful. All the six elements first mentioned are *absolutely essential*, however, and if only one of them be absent from the solution the increase in dry weight is greatly curtailed. Instead of increasing (in dry weight) from 1 to 138, the plant, in the absence of magnesium, increases only to 5·1; without calcium, only to 1·3; without potassium, only to 9·2 (compare Fig. 19, *II*); without iron only to 7·3 (in another experiment only to 3·3); without phosphorus only to 6·5; without sulphur only to 4·9; and only in a second experiment, where sulphur was omitted, did the increase reach the relatively high figure of 35·4.

Numberless experiments have been carried out with such nutritive solutions, all giving the same or similar results. [CRONE has obtained excellent results with the following solution: potassium nitrate, 1 g.; ferrous phosphate, 0·5 g.; gypsum, 0·25 g.; magnesium sulphate, 0·25 g.; made up to 1–2 lit. with water (1904, Ergebnisse von Untersuch. üb. d. Wirkung der Phosphorsäure auf d. höhere Pflanze, &c., Diss. Bonn). Compare BENECKE, 1904 (Bot. Ztg. 62, II Abt. p. 123).]

It has been found best to keep the solution slightly acid; if it be alkaline it is apt to react injuriously on the plant, save in the case of aquatic plants

both of higher and lower grade, which frequently thrive better in a weak alkaline than in weak acid solutions (BENECKE, 1898). In addition to the reaction the concentration and quantities are of primary import. In general we use a solution of 1–5 per cent. of salts; but NOBBE (1867) found that a 5 per cent. solution was injurious to barley, and was forced to use solutions of weaker concentration and to employ larger vessels. More recently, WORTMANN (1892) experimented with culture vessels of much greater capacity, holding about 25 lit. In such vessels plants grow remarkably well, and it is unnecessary to renew the culture fluids during the vegetative period.

Annual plants are especially well adapted for water-culture experiments, since rapid results may be obtained from them. Thus many grasses, Cruciferae, buckwheat, rape, linseed, and *Tradescantia*, as well as potatoes, may be employed with success. Indeed in the case of buckwheat it has been affirmed (NOBBE, 1868) that the plant grows better and shows a far greater increase in dry weight (from 1 to 4786) under such artificial conditions than when cultivated in soil in the ordinary way. A similar remarkable development is attained by oats, which, according to WOLFF (1868) show an increase from 1 to 2359.

Fig. 19. Buckwheat grown in water-culture solutions. *I*, normal; *II*, without potassium. (After NOBBE, from the Bonn Textbook.)

Many trees also, such as the oak, horse-chestnut, and alder, can be cultivated in aqueous solutions. It is not surprising, however, to find that not every plant proves a suitable subject for water-culture, since the method assumes a capacity for absorption on the part of the root under conditions which are far from natural—a capacity which the root of every plant does not possess. In those cases in which the water-culture method is unfavourable to the growth of the plant, the method recommended, especially by HELLRIEGEL (1883), may be adopted, viz. to employ a medium consisting of a quantity of sand which has been thoroughly cleaned by being heated to redness and boiled in sulphuric acid, and to which has afterwards been added the ingredients whose functions require investigation.

The culture methods used for the lower plants, Algae and Fungi, need not be studied here in detail, because they are either self-apparent or are, as in the case of seaweeds, still in want of improvement.

Collecting together all the results which have been arrived at as to which constituents of the ash in different plants are indispensable, we find that the six elements essential, according to BIRNER and LUCANUS, to the growth of oats, are also essential to all other Phanerogams, or, at any rate, to the majority of them, while to buckwheat (according to NOBBE, 1862) chlorine is also essential. Since, in other cases also, chlorine has been found to produce a favourable effect it may be added to the nutritive solution. The problem as to what special demands on inorganic materials individual plants may make will be discussed later on. It has been shown by BENECKE (1894–8) and MOLISCH (1895–6) that plants of low grade such as Fungi and Algae require fewer inorganic salts than Phanerogams, for calcium is not essential in their case, so that only five elements have to be considered as essential.

The plant requires a certain *definite amount* of each essential element. If too little of one element be present the plant is unable to develop healthily, even if the others be present in excess. This fact is sometimes expressed thus:—(Ad. Mayer, 1902, I, 323) *The nutrient present in minimum quantity gives a standard for the amount of production as a whole.* ('Law of the minimum.')

Despite the efforts of numerous investigators we are, unfortunately, still very much in the dark as to wherein the need for these five or six elements lies, and on taking stock of what little we do know we are brought face to face with the further problem as to the *combinations* in which these elements are employed by the plant. The significance of *sulphur* and *phosphorus* has been sufficiently well established, for they are, as we have seen, as essential constituents of proteids as carbon, hydrogen, oxygen, and nitrogen; sulphur occurs generally in such bodies, but phosphorus only in some of them, e. g. nucleins and many globulins. Further, it is by no means a matter of indifference in what combinations these elements are presented to the plant; it has been shown, on the contrary, that they must be presented in highly oxidized forms, such as sulphuric and phosphoric acids. Sulphurous and hyposulphurous are as useless as phosphorous and hypophosphorous acids, in fact, they are even poisonous to many plants; nor can sulphur and phosphorus be used in the elemental form. It may be noted, in passing, that only *one* essential element is absorbed by the plant in the elemental form, viz. oxygen. Further, sulphuric acid must be presented to the plant, united with a metal, although it is apparently a matter of indifference whether it be supplied as a sulphate of potassium, magnesium, or calcium, thus offering simultaneously a necessary metal, or as a sulphate of sodium or aluminium. We are, again, quite ignorant as to the region in the plant where such sulphates and phosphates are transformed or 'assimilated'. Schimper (1890) has shown that plants which absorb *abundance* of sulphate store it unaltered in many cells; if, however, only a small quantity of sulphate is available, it is, after entry, altered into a form which no longer gives the sulphuric acid reaction; no such reaction can be obtained from the young cells of meristem or from buds or pollen-grains. The same is true of phosphoric acid; it may be presented as a salt of potassium or sodium, and these compounds are more soluble than compounds with calcium, magnesium, or iron. In all situations where one fails to obtain the sulphuric acid reaction the phosphoric acid reaction is also wanting; at the same time, certain plants, e. g. the horse-chestnut, *Forsythia*, the onion, &c., are well known to store up large quantities of phosphate in old parenchymatous cells of the leaf (compare also Lecture XI). In addition to the proteids it may be noted that there are other sulphur-containing compounds in the plant which, however, owing to their limited distribution, need not be referred to here; they will be discussed later on in Lecture XVIII, where the special significance of sulphur in the vital economy of the sulphur-bacteria is treated of.

The third non-metallic element we have to consider is *chlorine*. It is employed only as hydrochloric acid and generally added to the solution as potassium chloride. As already mentioned, chlorine cannot be ranked as of the same importance as sulphur or phosphorus, for although of very general *service*, it is rarely directly *essential*. In addition to being present in buckwheat, as already mentioned, chlorine occurs (according to Beyer, 1869) in peas and oats, but as to its special significance in these plants we are entirely in the dark. It might be expected to play some part, more especially in plants which grow in soils containing common salt, but some of these plants develop perfectly well without any chlorine. We are ignorant also whether seaweeds can exist without this element.

Among the metals, *potassium* is absolutely essential, and it is immaterial with what acid it be united. Efforts have been made to replace potassium by one or other of the related metals, lithium, sodium, rubidium, caesium, but all these are

found to be quite unsuitable, and, save sodium, all are poisonous. Sodium, it is true, may to a certain extent act as a substitute for potassium, e.g. if the latter be present only in small amount. Under such circumstances the plant thrives better if sodium be provided than if it be not; a partial replacement of potassium by sodium may be conceded, but we must not forget that this does not apply in other cases, and certainly never when the principal functions are under consideration. The case appears to be otherwise in the lower organisms. The Cyanophyceae (according to BENECKE, 1898, p. 96) get on just as well with sodium as with potassium; in the lower Fungi potassium cannot be replaced by sodium or lithium, but BENECKE found a marked increase in dry weight when rubidium only was used, an increase which, when the rubidium was present in a certain concentration, was as great as when potassium was present in the nutritive solution. It is true that in this case there was a development of vegetative organs only, and no spores were formed, hence one may conclude that potassium cannot be entirely replaced by rubidium. Caesium behaves like rubidium. It is possible that traces of potassium were present with these metals as impurities, and so might influence the result; another explanation of this remarkable result will be given later (p. 88). Apart from such doubtful cases, we may say that potassium is absolutely essential. The function of potassium in higher plants may be deduced from the effect of its exclusion from water-cultures. SCHIMPER (1890) observed that in *Tradescantia* new organs containing potassium were still produced at the growing point, although potassium was excluded from the culture fluid. This is explained by the fact that the new leaves obtained it from the older leaves, which died off when potassium was removed. The new leaves were in every case, however, smaller and thinner, and in the end attained only minute dimensions. As the amount of potassium available from the dying tissues became less and less the growing point began to die also. This research proves conclusively that potassium is essential to the formation of the primordia of organs, whose size depends within certain limits on the amount of potassium available. Although not based on actual evidence it is still very probable that potassium plays an important part in the construction of the principal compounds which occur in protoplasm, more especially the proteids. The evidence for this belief is not as yet forthcoming in physiological chemistry, still any day may produce it.

What has been said of potassium is true also of *magnesium*. This metal cannot be replaced by any related alkaline earth; it is itself essential to every member of the vegetable kingdom. Water-cultures, from which magnesium has been excluded, give results similar to those which have no potassium. In this case, also, we are driven to believe that magnesium takes part in the construction of proteid, more especially since, according to SCHMIEDEBERG (1877), the proteid crystals of the brazil-nut consist of a magnesium salt of vitellin, and since GRÜBLER (1881) has proved that the crystallizable proteid of the gourd contains magnesium in no inconsiderable amount. Magnesium appears always so to be a constituent of chlorophyll.

The case is quite otherwise with *calcium*. Though most Algae and Fungi can do without it (according to BENECKE, 1898, it is essential in the cases of *Spirogyra* and *Vaucheria*), we must not jump to the conclusion that it is an important constituent of the proteids of protoplasm, although various important chemical compounds in individual groups of plants contain it. There are other grounds, however, against this view as to the significance of calcium in Phanerogams. To begin with, according to SCHIMPER, calcium is absent from regions where protoplasm is being formed and where potassium and magnesium are prominently present, e. g. at growing apices; on the other hand, it occurs in large quantities in older organs and especially in leaves. The appearance of *Tradescantia* when grown in a water-culture without calcium is

quite distinct from that in the absence of potassium or magnesium. During the first few weeks the growth appears healthy and the newly formed leaves are of normal size ; then *they*—but not the older leaves—begin to die off after the appearance on them of brown spots. SCHIMPER has shown that these brown spots are due to poisoning by oxalic acid, which cannot be neutralized owing to the absence of calcium, and he has drawn the conclusion that, *in general*, the neutralization of this acid is the function of calcium. PFEFFER (Phys. I, 427) points out, on the other hand, that those plants which do not form calcium oxalate, and they are by no means few in number, are as much injured by free oxalic acid or potassium oxalate as the others. More recently, PORTHEIM (1901) has succeeded in demonstrating that beans grown in a calcium-free soil become diseased, but that they showed the presence neither of oxalic nor of any other strong free acid. SCHIMPER'S hypothesis may be correct in individual cases, but it does not explain the general necessity for calcium, and we are bound to admit that its function has not as yet been discovered. In looking for a substitute for calcium, strontium would naturally first occur to us, but the researches of HASELHOFF (1893), undertaken to determine the capabilities of replacement of calcium by this metal, are not very convincing. BENECKE (1895, p. 521) found that strontium was poisonous to Fungi.

Our knowledge of the function of *iron* has been long believed to be much better grounded than that of the other constituents of the ash. Absence of iron in Phanerogams brings about the highly characteristic appearance known as chlorosis. Chlorosis consists in the young organs taking on a pale yellow or bleached appearance, as a consequence of which they soon die, owing to their not possessing chlorophyll, which we shall find (Lecture IX) plays so important a part in nutrition. For the development of the chlorotic condition in plants water-culture solutions from which every trace of iron has been carefully excluded are necessary. Even then chlorosis appears at first only gradually. The first leaves of the seedling are always green, because there is enough iron present in the reserves to supply what is wanted. As a matter of fact some plants with large cotyledons, such as the bean, are especially unsuitable for such experiments, because the amount of iron present in them is sufficient for the whole plant ; good results may, on the other hand, be obtained by using such plants as maize, buckwheat, or sunflowers. Pea-seedlings grown in iron-free nutritive solutions develop, according to MOLISCH (1892), three or four green leaves first of all, then one yellowish-green leaf; the remaining leaves, as well as the tendrils, are white. Such chlorotic plants may be again made green, as E. GRIS (1843) first showed, by permitting them to absorb an iron salt through the root, or by applying it directly to the chlorotic leaf. The cuticle of the leaf must, for the success of the experiment, be possessed of a certain degree of permeability, such as is exhibited, according to MOLISCH (1892) by *Helianthus*. Further, if greening is to take place the chlorotic organ must be young ; application of an iron salt to old chlorotic leaves has no effect.

For a long time chlorophyll was held to contain iron, and the appearance and disappearance of chlorosis in these experiments was accounted for by the presence or absence of that metal. The question as to whether or not chlorophyll contains iron has been reinvestigated by MOLISCH (1892), who has confirmed an older research of RAULIN'S (1869), showing that Fungi which have no chlorophyll, cannot do without iron ; BENECKE (1895) draws attention to the same fact. Another function, therefore, must be found for iron. It seems probable that, like potassium and magnesium, iron is necessary to the formation of protoplasm, and that its absence is followed by chlorosis in the higher plants as a *secondary* effect. Further, iron cannot be replaced by any other related metal such as manganese.

One element present in the nutritive solution alone remains to be con-

sidered, viz. *nitrogen*. When nitrogen in the form of nitric acid is *absent* no noticeable increase in dry weight takes place even though all the other salts be present, hence it should be mentioned here that nitric acid, in a form capable of being absorbed from the soil in water, is essential, although, not being found in the ash of plants, it need not be discussed here. It may be noted that one of the characteristics of the substances which we have hitherto been discussing is their capacity for *resisting heat*, but this characteristic is of no consequence so far as the plant is concerned ; nor is it of any importance whether, in ordinary combustion, the nitrogen is *given off* as a free gas or as ammonia, since in the plant the nitrogen is firmly combined and only very rarely escapes in the gaseous form. We will content ourselves with noting that nitrogen, in the same sense as sulphur, potassium, phosphorus, calcium, magnesium, and iron, is an essential food-stuff in every plant ; any further discussion of its characters would be premature (compare Lecture XI).

To sum up, we may say that we have clearly established a function for nitrogen, sulphur, and phosphorus, viz. that they undoubtedly take part in the formation of the living substance, and we have, further, good ground for the belief that to these elements must be added potassium, magnesium, and iron ; on the other hand, it may be said with certainty that this is, in general, not true for calcium. Scattered through the very voluminous literature on the subject, reaching from the time of LIEBIG to our own day, we find many statements, suggestions, and hypotheses as to the function of the inorganic salts. Thus, according to LIEBIG, the bases act as neutralizers of the acids—a fact which cannot be doubted—but it is not so easy to say why *special* metals should be required for this purpose. Again, it is stated that potassium is required for the formation of osmotically active bodies, that other elements render possible, or play a part in, the circulation of proteids, or in the construction of the cell membrane, of the nucleus, or the other organs of the cell. We must rest content with this brief summary of the literature and leave over any detailed criticism for the present, seeing that the various views above referred to have not been sufficiently established.

In addi ion to the *essential* constituents of the ash the plant also absorbs *non-essentials* from the soil, in greater or less quantity. Generalizations on this question are, however, scarcely valid, and although, also, very few plants are to be found which can subsist on the six elements mentioned above, there are others again which make specific claims on the soil. Buckwheat, which, according to NOBBE (1862), cannot fruit properly if chlorine be absent, may be cited as a striking example of the existence of such specific differences. It is necessary, first of all, to examine substances which occur only in certain plants. Thus it would not be surprising if it were proved that iodine was an important nutrient for marine Algae, or if it turned out that aluminium, which forms 22 to 39 per cent. of the ash of *Lycopodium chamaecyparissus*, *L. complanatum*, and *L. clavatum*, and yet appears in the minutest traces in most plants, including several other species of *Lycopodium*, has a special function to perform in these plants. [Large quantities of aluminium occur in species of *Symplocos* and *Orites* (CZAPEK II). JAMANO (Bot. Centrbl. 99, 2) found that aluminium was of service in the development of barley.]

Similarly lithium, which is, generally speaking, not only redundant but even poisonous, may be useful to those plants in which TSCHERMAK (1899) demonstrated its constant occurrence in plants taken from various situations.

We do know, however, that certain substances which appear to be absorbed in large quantity are yet actually superfluous, although they must not be looked upon as entirely functionless. *Sodium*, for instance, appears in almost all analyses quoted in the table on p. 80 in larger quantities than the indispensable iron. It may be assumed that this element has some duty to fulfil, though we cannot prove

it. It might serve, for instance, in place of another metal to neutralize acids and in the form of a salt act as osmotically in the cell-sap. Again we may note that *silicon* very rarely occurs in young organs or in seeds, although it is abundantly present as silica in the shells of diatoms and in horsetails and grasses (see p. 80, Nos. 10 and 11), and in the majority of cases is localized in the cell-walls of old stems and leaves. Its accumulation does not necessarily point to a use in metabolism, since its appearance may be due merely to withdrawal of the medium of solution. SALM-HORSTMAR held that silica was an essential constituent of the plant, but SACHS (1862) showed that maize could be grown satisfactorily in a silica-free water-culture. The evidence is, however, not quite conclusive, since the ash of maize plants grown in the 'silica-free' solutions still contained 0·7 per cent. of silicic acid (instead of 18–23 per cent.), which it had absorbed from the glass of the vessel in which the culture was made. Similarly, JODIN (1883) cultivated four generations of maize in silica-free solutions, one after the other, but he was not successful in completely excluding silicon, for in the second generation there was more silica present than sulphuric acid. On the other hand, some observers, e. g. SWIECICKI (1900) have endeavoured to show that silicic acid has a favourable influence on the plant. At present, all we can say is that the large quantities of silica present in grasses are certainly unnecessary, but that it has not been proved that they can get on equally well when silica is *entirely* absent. As to whether silica is of use or not in the Equisetaceae and Diatomaceae we are quite ignorant. Again, it is worthy of note that although silica may be quite superfluous from the *chemical* point of view it may be of great service to the plant in the *biological* sense. Our knowledge of these subjects, despite the amount of work which has been expended on them, is still very imperfect, and it is possible to defend the assertion that *all* the ash constituents have definite *functions* to perform, although these have not as yet been determined in all cases, and although these constituents cannot be considered as taking part in metabolic changes.

Under the circumstances it is unnecessary to present a complete enumeration of the 'non-essential' constituents of the ash. The occurrence of *manganese* may, however, be specially noted, as leading to the consideration of a new series of phenomena. It is not widely distributed in the earth, and yet is found, though only in traces, in very many plants. RAULIN (1869) has shown it to be instrumental in the development of Mould-fungi. Nevertheless it is certain that these organisms can exist for generations without manganese, and that it must not be looked upon as a *nutrient*. [See GÖSSL (Beihft. z. bot. Centrbl. 1905, 18, 1, 119) for a discussion of the distribution and functions of manganese.] Still more noticeable are RAULIN'S discoveries with regard to *zinc*, which have been recently completely confirmed by RICHARDS (1897). (Certain corrections of RICHARDS'S results have been made by A. RICHTER, 1901.) RAULIN showed that the addition of 0·0005 per cent. of zinc sulphate to a nutritive solution materially aided the growth of Fungi, and that a 0·003 per cent. solution of the same salt brought about a doubling of the plant's weight. The greatest effect was observed with this concentration, a further increase not only inhibited growth but acted injuriously. There are quite a number of substances which behave in a similar way, acting favourably in dilute solutions, but injuriously in stronger. Cobalt sulphate gives an optimum effect with a concentration of 0·002 per cent.; nickel sulphate acts best in a 0·033 per cent. solution. ONO (1900) found that an acceleration of growth took place after the addition of minute quantities of ithium nitrate, potassium arsenite, and sodium fluoride to Algae, and of mercuric chloride and copper sulphate to Fungi. But a poisonous effect does not always take place immediately, certainly not in the case of silicon, which, according to RAULIN and RICHARDS, acts beneficially. On the other hand, there are poisons which are injurious in small

doses and never accelerate growth, e. g. mercuric chloride, copper sulphate in Algae (Ono), copper sulphate in *Aspergillus* (Richter, 1901). How are such results to be interpreted? Raulin considered zinc and silicon as direct nutrients for Fungi, a view which cannot be accepted nowadays; it is more probable that Richards (following on the preliminary investigations of Pfeffer, 1895) is correct in holding that these substances act as stimulants and not as nutrients. Furthermore, on the presentation of the optimum amount of the stimulant, development in the fungus is no longer normal, but an *increase of vegetative growth* is induced and a *retardation of the formation of conidia*. The normal correlation of growth in the organs is also interfered with, and the organism as a whole ceases to thrive when such conditions are introduced. It is, as a rule, quite possible to differentiate *nutritive* from *stimulatory* materials, for when the nutritive substances, or only *one* of them, are carefully eliminated, the development of the organism comes to a standstill; when the stimulants are omitted, growth is retarded but is otherwise normal. This distinction is not readily made out in all cases; iron, for example, is a difficult element to deal with, because it is essential only in the minutest traces, and is possibly both a nutrient and a stimulant. Further, it must be remembered that such well-known food-stuffs as salts of potassium are tolerated by the plant only when in very low concentration, while in higher concentration they act injuriously owing to their osmotic action.

The facts which have now been put forward render intelligible a whole series of observations which were previously obscure. For example, take Benecke's observations on rubidium. We must bear in mind that the rubidium presented to the plant may not have been quite free from potassium as an impurity, so that we might regard the rubidium merely as a stimulant, while the traces of potassium might be considered as nutritive. Rubidium acts, in fact, like zinc sulphate; in relatively small amounts it acts directly as a poison and hinders the formation of conidia. Stimulus action further explains the favourable influence often observed on the addition of silica to a water-culture, perhaps also the good effect which carbon disulphide has on arable land previous to the beginning of vegetative growth, by the action of sodium fluoride on crops and possibly also that of copper on the higher plants. Copper is usually very injurious to plants, and Nägeli has shown that it is a deadly poison to *Spirogyra* even in a state of dilution so great that it cannot be chemically detected. Hattori has shown that 0·00005 per cent. is the extreme limit of concentration for the pea, and 0·000005 per cent. for the maize, above which injury ensues. All the same, sulphate of copper together with lime, under the name of 'Bordeaux mixture' has been used with success in combating diseases due to Fungi, and it has a further and unexpected effect, for plants sprinkled with the mixture grow more luxuriantly than control plants not so treated, provided that the latter are free from infection by the fungus. Vines and potatoes treated with copper show a greater development of chlorophyll and a more vigorous production of organic substance. Why syringing with this solution should have this effect cannot be explained at present, and the most varied views are held on the subject. The favourable effect of this mixture is claimed to be due to the lime; Aderhold (1899) recently advanced the view that it was due to the adulteration of this substance with *iron*, but it is also possible that *copper* was the active cause. At all events we must not draw the contrary conclusion that copper is not to be identified chemically in the leaves, since if copper be useful it must obviously enter in only in the very minutest traces. A full discussion of the copper problem is impossible here; a summary of our knowledge, along with new experiments, will be found in Bain, 1902.

In conclusion, we may mention that organic substances of various kinds may act in a similar way as stimulants, just as do the inorganic salts above mentioned. Thus in Richards's cultures cocaine and morphine acted as weak

stimulants, while amygdalin had a greater effect. These substances, however, certainly do not belong to the ash constituents of the plant, and for that reason we may stop at this point, returning to the question of stimuli later on, where we will be in a better position to make the subject intelligible.

Bibliography to Lecture VII.

ADERHOLD. 1899. Centrbl. Bakt.; II. Abt. 5, 217.
BAIN. 1902. Bullet. Agric. Exp. Station Univ. Tennessee, 15, Nr. 2.
BENECKE. 1894. Ber. d. bot. Gesell. (105).
BENECKE. 1895. Jahrb. f. wiss. Bot. 28, 487.
BENECKE. 1896. Bot. Ztg. 54, 97.
BENECKE. 1898. Ibid. 56, 83.
BEYER. 1869. Versuchsstat. II, 263.
BIRNER and LUCANUS. 1866. Versuchsstat. 8, 128.
BOUSSINGAULT. 1860. Chimie agricole (2nd ed.), 1.
GRANDEAU and BOUTON. 1877. Compt. Rend. 84, 129; Just's Jahresbericht, 1877.
GRIS. 1843. Cited by MOLISCH, 1892.
GRÜBLER. 1881. Journ. f. pr. Chem. 131, 97.
HASELHOFF. 1893. Landw. Jahrbücher, 22, 851.
HATTORI. 1899. Bot. Centrbl. 80, 171.
HELLRIEGEL. 1883. Beitr. z. d. naturw. Grundlagen d. Ackerbaues. Braunschweig.
JODIN. 1883. Annales d. Chim. et d. Phys. v, 30, 485.
KNOP. 1860. Versuchsstat. 2, 65 and 270.
KNOP. 1868. Kreislauf des Stoffes. Leipzig.
KNOP. 1884. Versuchsstat. 30, 293.
LIEBIG, J. 1840. Die Chemie in der Anwendung auf Agrikultur, 7th ed., 1862.
MALAGUTI and DUROCHER. 1858. Annales sc. nat. IV, 9, 222.
MAYER, AD. 1902. Agrikulturchemie, 5th ed., Heidelberg.
MOLISCH. 1895 and 1896. Sitzungsber. Wiener Akad. 104, I, 783; 105, I, 633.
MOLISCH. 1892. Die Pflanze in ihrer Bez. zum Eisen. Jena.
NÄGELI. 1893. Die ologodynamischen Erscheinungen. Basel.
NOBBE. 1862. Versuchsstat. 4, 318.
NOBBE. 1867. Ibid. 9, 478.
NOBBE. 1868. Ibid. 10, 1.
ONO. 1900. Journ. Coll. Sci. Imp. Univ. Tokyo, 13, 141.
PFEFFER. 1895. Jahrb. f. wiss. Bot. 28, 238.
PORTHEIM. 1901. Sitzungsber. Wiener Akad. 110, I, 113.
RAULIN. 1869. Annales sc. nat. V, 11, 93.
RICHARDS. 1897. Jahrb. f. wiss. Bot. 30, 665.
RICHTER. 1901. Centrbl. f. Bakt. II, 7, 417.
SACHS, J. 1860. Versuchsstat. 2, 22 and 224.
SACHS, J. 1862. Flora, 45, 52.
SACHS, J. 1882. Vorlesungen über Pflanzenphysiol. Leipzig.
SALM HORSTMAR. 1856. Vers. und Resultate üb. d. Nahrung d. Pflanze. Braunschweig.
SAUSSURE, TH. 1804. Recherches sur la végétation; Ostwald's Klassiker, 15 and 16.
SCHIMPER. 1890. Flora, 73, 207.
SCHMIEDEBERG. 1877. Zeitschr. f. physiol. Chem. 1, 205.
SCHRADER. 1800. Preisschrift über die eigentl. Erzeugung der erdigen Bestandteile in den Getreidearten. Berlin.
SENEBIER. 1800. Physiol. vegetal., Vol. 3.
SWIECICKI. 1900. Ber. aus d. landw. Instit. Halle, 14.
TSCHERMAK. 1899. Just's Jahresbericht, 27, 2, 188.
WIEGMANN and POLSTORFF. 1842. Ü. d. anorg. Bestandteile d. Pflanze. Braunschweig.
WOLFF. 1868. Versuchsstat. 10, 351.
WOLFF. 1871–1880. Aschenanalysen v. landw. Produkten. Berlin.
WORTMANN. 1892. Bot. Ztg. 50, 643.

LECTURE VIII

ASH. II

'THE mineral matters which are present in every plant, so far from being impurities, are quite as important constructive bodies as carbon and nitrogen.' This statement summarizes the chief results obtained in our last lecture. What was demonstrated there by experimental methods may also be established by observation of plants occurring wild and in cultivation. It may be proved without difficulty that the soil whence the materials of the ash are obtained has, quite apart from the water it contains, a most important influence on the development of the plant. Plants do not grow nearly so well in river sand, which is deficient in these mineral constituents, as in garden soil, nor are they so vigorous in their growth when the supply of garden soil is limited in quantity, as it is, for example, in pot cultivation (SACHS, 1892). These examples illustrate sufficiently clearly the vital importance of food-stuffs being supplied in sufficient *quantity* and of the right *quality*. Further inquiry into the nature of the contents of the soil, regarded as food-stuffs, and the manner of their absorption by the plant will serve only to emphasize this view of the problem.

Let us consider, first, soil as it occurs in nature formed by disintegration of the rocks, not such a soil as has supported many generations of plants and which has in turn received many of its constituents from them. The characters of the soil have been treated of more fully than we can do here by A. MAYER (1895) and by RAMANN (1893). [A new edition of RAMANN'S work, improved and enlarged, has been recently (1905) published under the title of 'Bodenkunde'.] Since the sedimentary strata have originated from the weathering and aqueous deposition of primitive rocks, all soils must in the long run have been derived from crystalline rock masses. Owing to the composition of these primitive rocks, the soil produced from them must be of varied chemical composition. An examination of granite as a source of soil gives us the following percentage composition (according to GIRARD ; compare MAYER, 1895, II, 12) :—

	Silicic acid.	Alumina.	Ferrous oxide.	Lime.	Magnesia.	Potash.	Soda.	Water.
I.	72·6	15·6	1·5	1·3	0·3	5·0	2·3	0·8
II.	68·6	14·4	5·0	3·9	0·4	2·8	3·4	1·1

Similar results are obtained when gneiss, mica-slate, and other rocks are analysed ; the differences between them lie merely in the *relative amounts* of the individual components, the same elements reappearing, but always in varying quantities. When we reduce such a rock to powder we obtain a soil containing the metals potassium, calcium, magnesium, iron, all important nutritive elements to the plant ; sulphuric and phosphoric acids, however, are not entirely wanting although they may be overlooked, owing to their occurrence in relatively small quantities (the sulphuric acid as gypsum and the phosphoric acid as apatite) (MAYER, 1895, II, 1); they are present as a matter of fact in not less quantity than in ordinary soil of cultivation.

If we now add to such a sample of powdered granite the one element which may be wanting, or present only in very small amount, viz. nitrogen, in the form of nitric acid, attempts to carry out culture experiments in it will lead to very poor results, because the bases are not united with hydrochloric, sulphuric, phosphoric, and nitric acids, as in our water-culture experiments, but chiefly with silicic acid forming for the most part insoluble compounds, more especially since the salts are present usually as double silicates. In consequence of the low temperatures which have prevailed in recent geological time a competition has taken place between the carbonic and silicic acids, which has resulted in the carbonic acid annexing the majority of the bases. These are carried away in the form of soluble compounds, and the rock in consequence is said to

weather; but wind and weather, oxygen and water, have much less to do with the process than carbonic acid, although it certainly requires the co-operation of water. The carbonic acid acts with varying rapidity on silicates of sodium, calcium, magnesium, and potassium, but is incapable of ousting the silicic acid from its union with aluminium. The primitive rocks consist of a mixture of different minerals whose capacity for weathering varies greatly. Looking at granite, for instance, we find that quartz and mica are remarkably stable, whilst the felspar (a double silicate of aluminium with potassium or sodium) weathers more easily. Through the action of carbonic acid, carbonates of sodium or potassium are produced from it, which are soluble in water, while on the other hand, aluminium silicate (caolin, clay), which, while retaining water, is entirely insoluble, is carried away by water in a state of suspension in very fine particles and redeposited as a clay soil. When the originally compact rock is in this way deprived of one of its ingredients, pits and cavities appear in it rendering it liable to fresh attack from the carbonic acid, as well as to certain physical effects of water which we need not refer to here. The net result of the whole process is the decomposition of the granite into a mass of felspar, quartz, and mica particles which, if they be still held together by the clay, are carried away by water and redeposited to form an *alluvial* soil. Such a soil is better for the plant than the original granite in two respects; in the first place, it is not compact, and hence plant roots can penetrate into it, and in the second place, it contains soluble constituents, which may be continually formed until the last particle of felspar has disappeared. The water, which such a soil holds, in virtue of its capillarity, as well as that which actually percolates through it, always contains substances in solution, although, it is true, only in limited amount. Analyses of streams and rivers which have their sources in primitive rocks teach us much on this subject (KNOP, 1868, 124) :—

One litre contains	Glacial rivers from crystalline Schist.		One litre contains	Mountain rivers from Granite and Gneiss.	
	Möll near HeiligenBlut.	Oetz.		Regen near Zwiesel.	Iltz near Passau.
Calcium carbonate	0·0084	0·00450	Sodium chloride .	0·0025	0·0059
Magnesium carbonate	0·0035	0·00005	Soda	0·0056	0·0043
Silicic acid . . .	0·0072	0·00868	Potash	0·0096	0·0058
Ferric oxide . . .	0·0010	—	Lime	0·0154	0·0092
Manganese oxide .	0·0032	—	Magnesia . . .	0·0026	0·0029
Alumina	trace	—	Ferric oxide . .	0·0009	0·0027
Magnesium sulphate	—	0·01301	Sulphuric acid .	0·0020	—
Sodium sulphate .	0·0009	—	Silicic acid . . .	0·0072	0·0095
Sodium chloride .	—	0·00043	Insoluble matter .	0·0018	0·0052
Suspended materials	0·0019	0·00853	Carbon-dioxide and organic matter .	0·0335	0·0450
	0·0261	0·03520		0·0811	0·0905
	Without carbon-dioxide and organic matter .			0·0476	0·0455

When we compare the contents of these natural soil waters with our culture solution, as regards the minerals they respectively contain, we find that the former solutions contain nearly 100 times less solid in solution than the latter, and further, that much of that solid is useless to the plant. It is also obvious that oats or maize will be able to thrive only with great difficulty in such a medium, seeing that phosphoric and nitric acids are present in such small quantities that they do not appear in the analysis. Soil water ought to exhibit, however, a composition similar to, and also show a concentration equal to, that of spring and river water, and we are thus at a loss to understand how it is possible for a plant to live in such a soil. Observation of natural conditions,

however, teaches us that plants of high organization which make considerable demands on the soil, such as oats and maize, never occur on such soils, but only plants which are unassuming in their requirements. In mountainous regions lichens are always the first plants to appear, and these organisms, although we must admit that qualitatively they require the same materials as Algae and Fungi (p. 82), are content with much smaller quantities, simply because they grow so very slowly. A higher plant, actively metabolic, would under such circumstances grow itself to death. As soon as lichens have effected the first settlement on such a primitive soil, mosses, ferns, and finally flowering plants quickly follow. In consequence, the substratum becomes less and less a natural soil of disintegration and more and more of the nature of an arable soil, for each generation of plants makes the soil fitter for its successors, notwithstanding the fact that it withdraws food materials from it. This follows from the fact that, in the first place, every plant gives off carbon-dioxide, which, as we have seen, has a disintegrating effect on the rock, and, secondly, that the dead parts of the plant, not only the roots in the soil, but also the leaves, twigs, branches, and stems formed above ground, ultimately reach the soil once more and decompose there. In consequence, their organic constituents will be either entirely destroyed and, amongst other things, carbon-dioxide will be produced, or such compounds as are more resistent to decomposition will be transformed into humus, to which the brown-black colour of the soil is due. Owing to the formation of carbon-dioxide in the process of decomposition of plant débris the air in the soil is always very rich in this gas ; WOLLNY (1897, 145) states that a minimum of 0·7 per cent. is present in the soil in winter time, and a maximum of 4·8 per cent. in summer, so that, in this respect also, vegetation has a marked effect on the rock constituents of the soil. The humus has in addition the power of altering the characters of the soil to a remarkable degree, both physically and chemically. From a *physical* point of view the humus particles, deposited between the mineral constituents, effect a loosening of the soil and, at the same time, increase its capacity for retaining moisture (p. 26). *Chemically*, certain special humin constituents are added. Since most of these are not considered as nutrients to the majority of plants, we need only say that they consist of little-known compounds of hydrogen, oxygen, nitrogen, and carbon, in part neutral in chemical reaction, in part showing the characteristics of acids. We shall have something to say afterwards as to the latter. In addition to the humin compounds humus contains, as well, the *ash constituents* of plants from which the humus has been derived, but in a form difficult to extract with water, without diminishing the absorption by the plant.

We have now to consider a phenomenon of the greatest importance to plant life, known as *soil absorption*, viz. the capacity of the soil to extract substances from their aqueous solutions.

In order to demonstrate this absorptive process we permit the filtration of a solution of indigo-carmine or yellow liquid manure through a layer of earth of a certain thickness ; we shall find that the fluids which escape are quite colourless. Were this power of absorption limited to colouring matters, the importance of the process, so far as plants are concerned, would be insignificant; but inorganic salts are also firmly retained by the soil, so that solutions of such bodies, after they have passed through the soil, have lost in concentration, and have also lost entirely certain constituents which they previously contained. Suppose we add to the soil one of the nutritive solutions mentioned on p. 81, we shall find that the potassium, calcium, magnesium, and phosphoric acid are retained, as well as ammonia and the less important plant nutrient sodium, but that the acids, other than phosphoric, i. e. nitric, sulphuric, and hydrochloric, are not. Absorption of the metals above mentioned belonging to the series of alkalis and alkaline earths takes place in different ways according as they occur

as salts or as oxides. In the following summary we will follow in general the statements of AD. MAYER (1895, II, 1) :—

1. The nitrates, sulphates, and chlorides of the metals above mentioned undergo transposition in the soil, so that the metals in these salts are exchanged for others occurring there. These latter go into solution whilst the former are absorbed. Thus potassium is more readily absorbed than ammonium, and that in turn more readily than magnesium, &c., so that we can affirm that in the series potassium, ammonium, magnesium, sodium, calcium, the succeeding element can be ousted by its predecessor from its combination ; more especially is it the case that frequently on the addition of salts of alkalis to the soil calcium-salts pass into solution. No rule is, however, without its exception, for, on the contrary, potassium can be turned out of combination by ammonium and sodium. This replacement of bases takes place most commonly if double silicates of aluminium are present in the soil which in addition to aluminium contains another base as well. The accessory base suffers replacement while the aluminium remains permanently united with silicic acid. Humates act like double silicates but less actively.

2. Oxides of alkalis and alkaline earths, as also their hydrated oxides, are absorbed in the first instance through the agency of humic acids, although no replacement takes place as in the first case. Double silicates and even pure caolin can act like humic acids.

3. As already noted phosphoric acid is apparently the only acid firmly retained, and that, too, whether it occurs as a salt or a free acid ; its retention is effected by carbonate of lime or even more firmly by hydrated ferric oxide.

The question of the *causes of absorption*, so often discussed by agricultural chemists, more especially as to whether absorption is to be regarded as a chemical or as a physical question, need not concern us ; what we know is that absorption takes place only in clay, chalk, or humus-containing soils, but not in quartz sand ; we are more concerned with the *effects* of absorption than with its causes. Soils formed by disintegration of rocks which are originally poor in salts suitable to act as plant nutrients, are thus gradually rendered more fertile, more especially since the substances absorbed by the soil are largely protected from being washed away by rain, although they are still capable of being taken up by the plant. Protection from removal by water is, of course, not absolute, but, according to PETERS (1860), it requires in most cases a very considerable amount of water to remove an absorbed substance from the soil ; for example, 28,000–36,600 parts of water are required to remove one part of potassium. Finally, it is of the highest importance that the individual substances so absorbed, if they be firmly combined, should be distributed in the soil in a state of *minute subdivision*. It will be seen later that in such a condition they are much more easily accessible to the plant.

Plants in the soil encounter an extremely dilute solution of nutritive salts, and, in proportion as they take up from it the more important of these salts for their own use, other salts previously absorbed will pass into solution. At the same time we must not assume that the plant's supplies are obtained *only* from the substances *in solution*; the plant is also able to make use of *solids* which it brings into solution. Although such solution phenomena are well seen in the lower plants, especially in lichens, still to avoid prolixity, we will confine our attention to the special characters of the root-system in the higher plants so far as they concern the absorption of minerals. In our earlier consideration of the root as the organ of absorption of water, opportunity was taken to speak of its mode of distribution in the soil. As a general rule the root meets with water and nutritive salts in the same place, and there are few adaptations known which are especially concerned in the absorption of salts only. Among these, the intimate fusion of the roothair and the soil particles may be especially noted, and that subject we

have not as yet considered in detail. Roothairs when grown in soil cannot maintain the symmetrical form they have in water, e. g. in *Hydrocharis*; in the course of their growth they encounter obstacles which they cannot avoid but round which they grow. In this way the roothair becomes irregular in form (Fig. 20), and, further, becomes so intimately connected with these obstacles, the minute particles of the soil, by means of its mucilaginous wall that we may, with every justice, speak of a *growth fusion* between them. In fact, the soil particles adhere to those regions of the roots that are covered with hairs so firmly that when the plant is pulled out of the soil these regions stand out prominently in contrast with the white apices which have *not as yet* developed roothairs, and also with the older parts of the root which have lost them. The soil, in a word, surrounds the root like a sleeve (Fig. 21). This intimate union of soil particles and roothairs renders easy the absorption of those substances which pass into solution on the breaking up of the soil, and this breaking up is in turn facilitated by certain excretions formed by the roothairs.

Fig. 20. Apex of a roothair in intimate union with soil particles. × 240. (From the Bonn Textbook.)

It has been known for long that roots in nature had a corroding effect on limestone. SACHS (1865, 189) demonstrated this fact by allowing plants to grow in a flower-pot in which he had placed a slab of polished marble covered with a layer of clean sand. The roots of the plant were thus able to grow over the marble, and, after several days or weeks, corrosion figures appeared on the plate corresponding to the distribution of the roots. SACHS found, as a matter of fact, that the course of the chief and secondary roots (e. g. of the bean) was mapped out, wherever they were in intimate contact with the marble, by a shallow rut about ½ mm. broad, bordered by a hazy and indistinct roughness in certain places indicative of the presence there of roothairs. He obtained similar results by employing dolomite, magnesite and osteolite, so that magnesium carbonate and calcium phosphate are as capable of suffering dissolution by roots as calcium carbonate. For a long time it was held that the roots gave off free organic acids, and that these acids were the cause of the formation of corrosion figures; more recent research has shown, however, that corrosion figures are due primarily to the action of *carbonic acid*. CZAPEK (1896) employed in his researches plates of plaster of Paris artificially compounded with the mineral whose solubility he desired to investigate, using them in the same way as SACHS did plates of marble. The two substances were pounded into a paste with distilled water and then spread over a glass plate; in this way a very fine flat surface was obtained for experiment, quite as effective as a polished plate of natural rock. By this means, CZAPEK established that plates of carbonate and phosphate of lime were corroded by roots while aluminium phosphate plates were not affected, and it may be concluded that quite a number of organic acids, those, in fact, in which aluminium phosphate is soluble, take no part in the formation of corrosion figures; excluding these acids we have only to consider carbonic, acetic, propionic, and butyric acids. The brown coloration given to congo-red demonstrates that carbonic acid at least must be considered as an agent in the formation of corrosion figures, since the other acids

Fig. 21. Seedling of white mustard. *I*, taken direct from the soil with adhering soil particles; *II*, after being washed. (After SACHS, Lectures on Plant Physiology.)

mentioned give a blue reaction with congo-red. [PRIANISCHNIKOW has shown that CZAPEK'S conclusions are by no means above criticism (Ber. d. bot. Gesell. 1904, 22, 184). At the present moment we have no certain knowledge as to what acids are given off by the root. More recently, PRIANISCHNIKOW (Ber. d. bot. Gesell. 1905, 23, 8) has proved that phosphates which are soluble with difficulty in presence of salts of ammonia are available for the nutrition of plants, in proportions quite different from those available when potassium nitrate only is present.]

It will be shown later that all plant cells produce abundant carbonic acid, and its presence in sufficient quantity in this relation cannot be doubted. Again, from the sharpness of the outlines of the corrosion figures it has been concluded that they must have been produced by a non-volatile acid; 'carbonic acid,' as SACHS says, 'penetrates freely into the interspaces in the soil, and hence one might expect to find corrosion in regions at some distance from the root.' But SACHS'S conclusions cannot invalidate CZAPEK'S view, viz. that the carbonic acid alone produced corrosion in the substances referred to; for one must remember, in the first place, that carbon-dioxide does not occur as a gas, but in solution in water, and in the second, that the water with carbon-dioxide in solution is markedly present in the cell-walls of the root epidermis, and does not readily exude therefrom. If the solution be in intimate contact with the minerals, and if the dissolved particles be at once absorbed into the interior of the cell, in course of time a quite observable effect will be produced.

Although we may look on the *carbonic acid* as the factor concerned in the formation of the corrosion figures on certain kinds of rock, and although, further, we have already ascribed to this substance, apart from the plant, an important rôle in the disintegration of rock, we are not entitled to affirm that the root is unable to excrete other substances which may be instrumental in opening the soil up. As we have already said, it has been generally accepted that the root gives off organic acids, and this view was held to be supported by the fact that organic acids could be demonstrated in the cells of the root and by the red colour often given to litmus paper when roots were pressed against it. To CZAPEK we owe a reinvestigation of this problem. He has confirmed the observation previously made of the excretion of minute drops from roots grown in spaces saturated with moisture, and has further shown that, as in the case of subaerial hydathodes, this phenomenon takes place only when the plant as a whole is turgid. CZAPEK also found that these drops gave a *neutral* reaction. Again, CZAPEK has grown roots in a minimum amount of water or on small pieces of filter paper, and after a time has submitted the water or filter paper to microchemical analysis. He finds potassium and phosphoric acid not infrequently present in quantity, smaller amounts of magnesium and chloride, and traces of calcium. The reaction of these fluids is on the whole *acid*, owing to the presence of acid-potassium phosphate, in other cases of acid salts of formic acid and (only in one case) of oxalic acid. [According to PRIANISCHNIKOW (1904, Ber. d. bot. Gesell. 22, 184), the excretion of phosphates is quite possible in seedlings, since these bodies are plentifully produced in the process of decomposition of proteids; this, however, is not the case in mature plants.] These bodies must have been excreted from the uninjured root-cells. Looked at scientifically these investigations are not above criticism, for it may be assumed that cell-sap from dead roothairs and from the dead cells of the root-caps was present in the fluid examined, and that in the experiments with filter paper injury was done to the roothairs in the process of cutting off of the rootlets, so that, in both cases, the occurrence of the substances referred to above might be explained without assuming that they were excreted from *living* cells. At any rate it is important to note that the reaction of this sap is acid as opposed to the result of CZAPEK'S investigations on the drops excreted from roothairs.

It may be further noted that the acids arising from the dead roothair

may very well play an important part in the dissolution of soil if these cells be sufficiently numerous. As to the amount of root-cap production we have certainly no accurate information, but our knowledge as to the roothairs is more complete. We know that they live only a few days and are replaced by new hairs formed behind the apex.

The fact that substances escaping from the plant play some part in the dissolution of the soil must be always borne in mind, since it is certain that the plant can extract from the soil much more in the way of nutrient material than we can with the aid of water holding carbon-dioxide in solution. Thus, e. g., LIEBIG (1862, 2, 108) says :—

'A young plant of rye, grown in a fertile soil, is often capable of developing into a tuft of thirty to forty shoots, each with an inflorescence bearing a thousand or more grains, and yet it obtains its nutriment from a volume of soil which, after prolonged washing with pure water, or water containing carbon-dioxide in solution, yields up not one hundredth part of the phosphoric acid and nitrogen, and not one fiftieth part of the potassium and silicic acid which the plant has absorbed from the soil. Under such circumstances how is it possible for the plant to obtain all the materials found in it by merely dissolving them in water?'

In order to obtain some idea how much nutrient material is available in the soil, agricultural chemists employ a dilute (1 per cent.) solution of citric acid instead of a solution of carbonic acid; since it has been shown that the plant is able to absorb as much material as it would do if it excreted weak citric acid. What the materials are which act as solvents, and which are given off by the plant, and whether these spring from living cells or from dead, are matters still to be made out. The corrosion figures are formed on natural or artificial plates only when the substances of which these plates are made possess relatively great solubility; moreover, a root, without forming obvious corrosion figures, can dissolve a considerable quantity of material if it be allowed to operate on a sufficiently large surface; these are undoubtedly the conditions which occur in nature. Further we must always remember that acids may be excreted only in the presence of definite substances, amongst which, perhaps, aluminium phosphate must not be classed. In this relation, CZAPEK has shown that acid-potassium phosphate may be of service in consequence of the *decompositions* which it excites outside the plant, e. g. when it reacts with neutral salts of the strong acids, and so gives rise to small quantities of mineral acids.

Attention has been drawn to the rapid death and constant renewal of roothairs and, in addition to the close union of roothairs and soil particles, this further point is of special importance in relation to the absorption of nutritive salts from the soil. Numberless roothairs develop from day to day on a large plant, which penetrate into new masses of soil, grow round the particles, taking nutritive salts from them, and rendering naturally insoluble particles soluble. Thus an ever-increasing area of soil becomes available. Since after each absorption of soil-water at any definite spot a movement of water takes place, tending to produce once more an equilibrium, it might be held that this continual acquisition of new soil particles was of little moment in the absorption of water and substances dissolved in it; but migration of solid bodies is out of the question, and hence the intimate union and constant renewal of roothairs is of the greatest importance. Although the roothairs are the ordinary organs of absorption of nutritive materials, there are many plants which normally possess none. Plants which develop roothairs in ordinary soil, as a rule, do not do so as a rule in water-cultures. In these cases the nutrients are absorbed either by means of the general epidermal cells or—and this is a very frequent method—by the aid of Fungi which live both on and in the root (compare Lecture XIX). In normal roots, also, the young epidermal cells, which have not as yet developed roothairs, are capable of absorbing nutrient substances (KNY, 1898).

Finally, a third point may be drawn attention to. Many investigators

have shown that roots branch much more freely in soils which contain abundant food materials than in those which are poor in them. Nobbe (1862 and 1868) has shown this by cultivating clover and maize in a soil which consisted throughout of the same basal material, but with the alternate layers saturated with a nutritive solution. Experiments on this subject were also made by Thiel (quoted by Sachs, 1865, p. 178), and more recently by Höveler (1892), who employed soils which consisted of alternate layers of sand and humus.

Having now determined in what condition the plant finds its food-stuffs in the soil, and how it absorbs these with the aid of its roots, there remains for us to study how variations in the chemical composition of the soil influence its occupation by plants. We have already considered how the primal colonization of a rock takes place, and how it is transformed into a soil under the influence of plants which are easily satisfied. It has further been shown how the humus compounds which arise from the decomposition of not only plant but also animal remains, add to the fertility of the soil. Soils occur in nature, however, which are quite free from vegetation and that for very varied reasons. The great resistance presented by some minerals to decomposition must be considered first, and an example of such is seen in lava, which becomes covered with vegetation only extremely slowly (compare Treub, 1888, on Krakatao, and Schimper, 1898, 200, on Gunung Guntur). On the other hand, although it rarely happens, a rock may weather, but does not contain all the elements required by the plant, or, again, it may contain too great a proportion of mineral salts (e.g. sodium chloride, &c.) which interfere with the introduction of plants, or it may be deficient in water. Such regions of the earth which are destitute of vegetation we term deserts. The greater part of the earth's surface is capable of supplying the plant with all the chemical compounds necessary and in quantities sufficient for its growth, and for that reason it is covered with vegetation, but this vegetative covering takes on very varied characters in different regions. In so far as we are able to understand the causes of this variation, we may refer to climate and soil as the most important determining factors in plant distribution. Here we are naturally concerned with soil only, and we may draw attention to a fact with which we have been long acquainted, that a seashore whose soil contains salt in large quantity has quite as characteristic a flora as inland regions with, say, a lime soil, which exhibit plant societies other than those shown by a sandy soil poor in lime, or by primitive rock. We distinguish in geographical botany between plants which are confined strictly to soils with definite characters, and such as are able to thrive in various kinds of soil; the former are 'local' in distribution, the latter 'indifferent'.

Thus there is quite a number of *halophytes* which in nature occur by preference or exclusively on a soil which contains a large quantity of sodium chloride, such, for example, as a seashore, where one usually finds as much as 3 per cent. of common salt. So far as we know common salt, however, performs no special function in their metabolism other than it performs in the rest of the vegetable kingdom, for these halophytes can exist in soils containing a mere trace of this salt or none at all. The point where halophytes differ from other plants lies in their capacity for *tolerating* quantities of sodium chloride which are directly injurious to non-halophytes. In virtue of this power they are able to exist in places from which other plants are debarred, while in ordinary soils they, for the most part, give place to non-halophytic types. The injuries inflicted on ordinary plants owing to the presence of an excessive amount of salt in the soil depend, in the first place, on the osmotic action of the concentrated solutions in the soil, and in the second place, on the difficulty of bringing about absorption of water; further, common salt, when absorbed, has certain, to all appearance, after-effects which are not as yet perfectly understood. Halophytes overcome the greater difficulty of absorption by being extremely economical in

their use of water, and by reducing transpiration by special methods which are naturally adapted to the special conditions under which they live. It is of interest to note that some of them have discovered a means, i. e. by hydathodes, of getting rid of excess of salt, and thus of preventing a superabundant accumulation of sodium chloride in their tissues (compare p. 58).

Our acquaintance with the causes which determine why many plants prefer soils *rich* or *poor* in lime is at present much less complete. It is obvious that the occurrence of plants in either situation is not directly connected with the need of the plant for calcium as a food-stuff, for no soil is so poor in calcium that it cannot obtain all that it requires from it. The so-called 'calciphobous' plants require calcium just as much as the 'calciphilous' plants, and they do, as a matter of fact, absorb considerable quantities of it from the slate or primitive rock on which they live. The solution of the question is rendered all the more difficult inasmuch as one and the same species cannot live equally well in all places. Only a few plants appear *constantly* to avoid lime soils, e. g. *Sphagnum* and certain other aquatic mosses, the majority of Desmidiaceae, and, among Phanerogams, *Sarothamnus scoparius*, *Castanea vesca*, and *Pinus pinaster*. In regard to the last-mentioned plant, VALLOT (1883) has made many investigations which show how exclusive it is in its choice of a soil. Generally speaking, soils in which it is said to thrive, and which contain more than about 3 per cent. of lime, are found on closer investigation to exhibit local conditions (e. g. oases of rock poor in lime) which render its existence possible. The experiments of BONNET (VALLOT, 1883, p. 202), made at Dijon, are of great interest, confirmed, as they have been, by MANGIN (according to ROUX, 1900, p. 131) at Besançon. The chestnut flatly refused to grow there, and yet it was possible to cultivate it easily when it had been grafted on the calciphilous oak. The reason is probably that the root is injured by the excessive amount of lime in the soil, and in support of the correctness of this view we may draw attention to the frequently cited behaviour of *Sphagnum*, as well as of plants which occur in its company, e. g. *Drosera*. After being watered with a solution containing lime these plants come to grief very quickly; solutions of lime salts act as poisons to them. According to ÖHLMANN (1898), watering *Sphagnum* with calcium sulphate and calcium nitrate is less harmful than treating it with calcium carbonate. ÖHLMANN also says that a 0·05 per cent. solution of calcium carbonate kills it in from twenty-two to thirty-two days, and that a 0·15 per cent. solution is fatal in fourteen to twenty-four days. More recently, GRÄBNER (1901, p. 112) states that WEBER cultivated *Sphagnum* on chalk successfully. A final decision on this question must be postponed until further investigations have been made. [SOLMS LAUBACH (1905, Die leitenden Gesichtspunkte einer allg. Pflanzengeographie, p. 122) has offered a more accurate analysis of WEBER'S results, whence it would appear that *Sphagnum* is certainly injured by lime, since it can scarcely live in company with other plants which require that mineral. GRÄBNER'S results obtained by cultivating *Sphagnum* on chalk apply only to pure cultures.] CORRENS'S (1896) observations on *Drosera* (compare Lecture XXXVIII) also point to a directly *injurious* effect of lime. How limited our knowledge is as to the function of lime in plant life, is evident from the investigations which A. ENGLER has recently (1901) made on the distribution of *Castanea* in Switzerland. Although we may consider this tree in general as markedly 'calciphobous', it occurs, according to ENGLER, on sandstones and marls rich in lime, and which possess a large proportion of potassium. ENGLER believes that a *great need for potassium, and not the absence of calcium*, determines in general the occurrence of *Castanea* on siliceous soils. It must certainly not be overlooked that in the case of plants of lower grade such as *Sphagnum*, it may be possible that calcium, as in the case of Algae, is not an essential food-material, while in the case of the higher plants such a supposition is less probable.

Although further investigations may not confirm the view that carbonate of lime is directly poisonous to higher plants, still they may establish that this substance has an indirect effect such as may be deduced from the experiments of FLICHE and GRANDEAU (1873). These investigators analysed the ash of trees which had been grown in normal soil and compared it with that of other specimens which had passed a miserable existence in a soil which was rich in lime. The result of this comparison was to show that the ash of those grown in a siliceous soil contained 40–45 per cent. of lime, while in those from a lime soil, the percentage rose to 56–75; at the same time the absorption of potassium was much reduced (from 16–22 per cent. down to 4–6 per cent.). It is conceivable that the diminution in the absorption of potassium is due to the fact that the calcium carbonate rapidly neutralizes the acid excretions of the root, and so interferes with its disintegrating action on rock material naturally difficult of solution. In addition to the diminution in potassium there is a scarcity also of magnesium and iron in plants grown in lime soils. SCHIMPER (1898, 110) attributes the feeble development of siliciferous plants on lime soils to the deficiency in *iron*. In support of this view one may cite the fact that calciphobous plants become chlorotic on lime soils (ROUX, 1900), and that this chlorosis (according to a verbal statement of Professor STAHL) can be set right by spraying with a solution containing iron. [BENECKE has obtained marked chlorosis in presence of iron when the water-culture gave an alkaline reaction (Bot. Ztg. 1904, 62, II, p. 124).]

A complete explanation of the *aversion* of many plants to lime is not at present available; we must be content with the suppositions advanced above. Nor are we better off in regard to our knowledge of the reasons for the *fondness* for lime exhibited by other plants. The view once held that these plants avoid contact with silicic acid need not be considered. That they can *endure* the presence of more lime than others can is obvious, but it is not so apparent what *use* they make of it. THURMANN has performed a useful service in demonstrating the *physical* differences between lime and sandy soils, especially the deficiency in water in the former and its abundance in the latter, and has suggested this as determining questions of plant distribution. According to THURMANN, 'calciphilous' plants are xerophytes, 'calciphobous' forms are hygrophytes, and it is quite true that we do meet with plants in the most varied regions which usually occur on chalk or, exceptionally, on primitive rock under very dry conditions. THURMANN, for example, has emphasized the fact that quite a series of calciphobous plants in Southern France also occur on primitive rock. This, however, must depend not only on the amount of water present, but on the sum-total of the physical characters of the soil, and amongst these the conditions of temperature at all events must be especially taken into account. These conditions have been specially investigated in recent times by WOLLNY (1898). It has been shown that a quartz soil changes most rapidly with alterations in the temperature of the air, then more slowly clay, lime, and magnesia soils respectively, and, finally, humus soils change most slowly of all. Chalk soils moderate the extreme temperatures of the air, being cooler in summer and warmer in winter than sandy soils.

NÄGELI (1865) has shown that it is as yet impossible to explain completely the distribution of plants by reference only to the chemical and physical characters of the soil; this he has proved in a classical treatise in which he draws attention to two new factors, hitherto entirely disregarded, which take part in determining the distribution of plants on the earth's surface. He starts from the fact, already referred to above, that a plant may be local in distribution in one district and indifferent in another, or that one and the same species may be calciphobous in one region and calciphilous in another. NÄGELI's well-known researches were performed on *Achillea atrata* and *Achillea moschata*, and we will take these also as our illustrations. NÄGELI found both species extremely restricted in distribution in

the Heu-Thal; *atrata* on chalk and *moschata* on slate. 'Wherever the slate passes into limestone, *moschata* at once stops and *atrata* begins.' In other places, however, where only one species occurs, it appears quite indifferent as to whether the substratum be slate or limestone. So long as only one is present both plants are indifferent as to the soil, but when both occur in company they at once become confined to special areas; they adapt themselves markedly to the *chemical* nature of the soil and are indifferent to the *physical* conditions, for they thrive equally well in wet places and dry places, on humus, on sand, or on rock. The mutual exclusiveness of these species of *Achillea* can be explained only by assuming the influence of concurrence or of the struggle for existence between nearly related species as the effective cause. Each species lives only on a soil where the conditions are better fitted for its existence. NÄGELI'S experiments do not demonstrate wherein lies the reason for *Achillea atrata's* preference for limestone and *moschata's* preference for slate. It may be concluded, however, that this preference is quite limited, and yet it may be sufficient to determine, in a state of nature, the existence or non-existence of the species. We are also familiar in our own country with plenty of examples of rapidly spreading American weeds which are capable of killing our native plants, and even entire floras, in a very short time; but we are quite ignorant as to wherein lies their power of intruding and ousting out the native flora. A glance at our cultivated plants is sufficient to prove to us that organisms, when withdrawn from the company of others, are able to exist under conditions which they cannot tolerate in the wild state. Although we are unacquainted with the *reasons* why *Achillea atrata* gains the ascendancy in one case and *Achillea moschata* in another, still we must accept the fact that association is a factor of the highest importance in determining the distribution of plants on the earth's surface.

NÄGELI has drawn attention to another important factor to account for the irregular occurrence of plants which we have space only to glance at in passing. A plant may be *absent* from an area, notwithstanding the fact that the chemical and physical conditions of the soil, the plant society, and the general climatic features are favourable, simply because none of its seeds have as yet been distributed to that district (Historical Plant Geography).

We must content ourselves here with this brief outline; further details will be found in the works of SCHIMPER (1898) and ENGLER (1879). *One* point at least is clear, viz. that these problems of plant geography are exceedingly complicated and cannot be settled *off-hand*. In fact, the unfortunate craze for looking for *one cause* instead of *several* only tends to obscure the real issue. When, in the future, accurate researches on this question come to be pieced together, other factors than those we have alluded to will, doubtless, be discovered. One result, however, these researches certainly will have, they will tend to discourage the forming of *summary* conclusions and draw more pointed attention to the details in *individual* cases both as regards the plant and also the soil, and so, doubtless, lead to the discovery of far more individual and specific differences than could be expected from the older researches on the subject.

In addition to salt, lime and siliceous soils, reference must in conclusion be made to *humus* soils, which are characterized by supporting special plant communities. We shall return to this subject later (Lecture XIX).

Before we finally leave the consideration of the mineral constituents, we will glance at cultivated plants as they are found in our fields and woods, and at the same time gain a yet greater appreciation of the fundamental importance of the minerals present in the soil. In nature, the covering of an area of soil by vegetation leads, as we have seen, to its enrichment in nutritive substances, since each plant on its death gives back to the soil what it took from it, and in a form also which other plants can easily appropriate. It is true each material

particle does not return to the identical clod from which it was derived, for wind and water displace many a leaf and twig, a storm may remove an entire tree, and even an entire wood with its wealth of mineral matter may be transported far away by an avalanche. Though such removals are the exception in *nature*, in *husbandry* they are the rule. When the ripe plants are *harvested*, fruits, leaves, and often stems as well are removed from the field; the roots alone remain where the plant grew. Although each plant contains only a *small* quantity of ash, the total amount is very considerable when an entire field is taken into account, and, according to EBERMAYER (1882) 200–300 kilg. per hectare of mineral substances are withdrawn when the harvest is reaped, about half of which is nitrogen, the remainder consisting of the other constituents of the ash. When we remember that this goes on year after year, we can easily understand how in a short time all the nutrients in the soil that are capable of absorption must disappear, and so in the end plant-life is possible only if the soil still contains constituents capable of decomposition. But decomposition of soil never takes place rapidly enough for cultivated plants to obtain the amount of inorganic nutrients they require. The soil, in other words, becomes *exhausted* by continuous cultivation, but is not on that account lost to the agriculturalist for all time, for it can be rendered fertile once more, and even improved by artificial means and by employing appropriate applications. Since all cultivated plants do not impoverish the soil in the same way, one type demanding, for example, more potassium, another lime, one may economize the soil by *rotation of crops*. During the period, for example, a calciphilous plant grows in a field the soil has time to enrich itself in potash by weathering, so that in the following year the conditions are favourable for the development of a plant which makes a demand on potash. Since, however, many substances, e. g. phosphates, always occur in the soil only in small quantities, and since all plants require a good deal of them, one cannot employ this method of rotation *exclusively*. A second method consists in leaving land to lie *fallow*. In this method fields are not made to support a crop continuously, but the land is allowed to be idle, weeds are permitted to grow on it, and these are subsequently ploughed in, thus adding to the humus constituents of the soil. By far the most important method is, however, the *addition of nutritive salts* to the soil, directly replacing what has been lost. This method is termed *manuring*, and was practised in husbandry long before people had learned its inward significance. The addition to the soil of the dung of animals mixed with the straw, as well as the general sewage of a town, naturally replaces in the soil a *part* at least of the mineral substances taken from it. LIEBIG showed that the chief value of these bodies lay in their *inorganic* constituents, so that we can understand how it is possible to improve or supplement such natural manures by artificial combinations.

Artificial manuring plays a great part in modern agriculture, and consists in the addition to the soil of potash, lime, and phosphoric acid, apart from nitric acid, of which we shall speak later. A sentence on this subject must suffice at this point. Lime occurs so abundantly in nature that one need never fear a deficiency of that mineral in agriculture. It is otherwise, however, with potash and phosphoric acid. The former is manufactured in great quantities at the potash works of Stassfurt and Leopoldshall in the form of karnallite, cainite, and sylvinite, and of these cainite ($KCl.MgSo_4.3H_2O$) is specially important, as it contains not only potassium but also magnesium in the form of sulphate, also a plant nutrient. As a source of phosphoric acid, 'Thomas-slag,' a by-product in the smelting of ores containing phosphorus, holds a foremost place. It is true that the phosphoric acid occurs in the form of a tricalcic salt [$Ca_3(PO_4)_2$], and that this is insoluble in water. Since, however, the manure is laid on the field in an exceedingly fine state of subdivision in the form of the

so-called 'Thomas-slag-powder', the plant is easily in a position to take up the necessary phosphoric acid. This mention of cainite and Thomas-slag-powder by no means exhausts the artificial manures used by agriculturalists but to give further detail would take us too far; reference must be made for such information to agricultural literature, e. g. AD. MAYER, Agricultural Chemistry, 1895.

Bibliography to Lecture VIII.

CORRENS. 1896. Bot. Ztg. 54, 21.
CZAPEK. 1896. Jahrb. f. wiss. Bot. 29, 321.
EBERMAYER. 1882. Physiol. Chemie d. Pflanzen. Berlin.
ENGLER. 1879–82. Versuch einer Entwickelungsgesch. d. Pflanzenwelt. Leipzig.
ENGLER, ARNOLD. 1901. Ber. Schweiz. bot. Gesell. 11, 23 (Bot. Centrbl. 89, 269).
FLICHE and GRANDEAU. 1873. Annales d. chim. et d. phys. IV, 2.
GRÄBNER. 1901. Die Heide Norddeutschlands. Leipzig.
HÖVELER. 1892. Jahrb. f. wiss. Bot. 24, 294.
KNOP. 1868. Der Kreislauf des Stoffes. Leipzig.
KNY. 1898. Ber. d. bot. Gesell. 16, 216.
LIEBIG. 1840. Die Chemie in ihrer Anwendung auf Agrikultur, 7th ed., 1862.
MAYER, AD. 1895. Lehrbuch der Agrikulturchemie, 4th ed., II, 1. Bodenkunde. Heidelberg.
NÄGELI. 1865. Sitzungsber. Münch. Akad. (Bot. Mitteilungen, 2, 1).
NOBBE. 1862. Versuchsstationen, 4, 217; 1868, ibid. 10, 94.
ÖHLMANN. 1898. Veg. Fortpfl. d. Sphagnaceen nebst ihrem Verh. gegen Kalk. Diss. Freiburg (Schweiz). Braunschweig.
PETERS. 1860. Versuchsstationen, 2, 135.
RAMANN. 1893. Forstliche Bodenkunde u. Standortslehre. Berlin.
ROUX. 1900. Traité des rapports des plantes avec le sol. Montpellier.
SACHS. 1865. Handbuch d. Experimental-Physiologie. Leipzig.
SACHS. 1892. Flora, 75, 171.
SCHIMPER. 1898. Pflanzengeographie auf physiolog. Grundlage. Jena.
THURMANN. 1849. Essai de phytostatique appl. à la chaîne du Jura.
TREUB. 1888. Annales Jard. bot. Buitenzorg.
VALLOT. 1883. Rech. physico-chimiques s. la terre végétale. Paris.
WOLLNY. 1897. Zersetzung d. organischen Stoffe u. d. Humusbildungen. Heidelberg.
WOLLNY. 1898. Forschungen a. d. Geb. d. Agrikulturphysik, 20, 133.

LECTURE IX

THE ASSIMILATION OF CARBON BY AUTOTROPHIC PLANTS. I

GREEN plants grown in nutritive solutions show a marked increase in dry weight (p. 82), but analysis of the dry substance of such plants shows that the predominant constituent is not the *mineral matter* absorbed from the solution, but *carbon*, of which, as a matter of fact, one half of the dry weight consists. Carbon occurs as a component of nearly every compound found in the plant, and the *number* of these compounds depends chiefly on the fact that carbon is able to unite with other elements in endlessly variable proportions. In one sense we may look on carbon as the *most important* material in plant nutrition, although it must be remembered that the minerals are every whit as essential as the carbon. Under these circumstances it may at first sight appear extraordinary that we have *not* introduced carbon into our culture solutions, or at least have not done so intentionally.

When we inquire whence plants obtain their carbon, we discover that the uniformity of method which prevails among them in regard to the mode of absorption of the constituents of the ash does not hold good here; there are fundamental differences between different types of plant life in this respect, some absorbing carbon in the *inorganic* form and transforming it into *organic* compounds, others making use of carbon solely in the *organic* form. The

former obtain *all* their nutriment directly from the inorganic world, and, so far as their nutrition is concerned, are thus entirely independent of other organisms; such forms we may speak of as *autotrophic*; the other we designate *heterotrophic*, and without the aid of autotrophic organisms their existence is impossible. From this it will be at once apparent how important a part is played in nature by autotrophic plants, and for that reason they claim the first place in our attention.

Organic compounds of carbon are not available for the support of plants grown in typical water-culture solutions, because the *air* contains none, or, at all events, quantities so minute that their presence may be entirely neglected, and because the *nutritive solution* in which the roots are immersed consists of inorganic salts only. Since maize, buckwheat, and many other green plants thrive just as well in such water-cultures as in the soil, it follows that the organic materials occurring in ordinary soils are either not absorbed at all or that they are at least unessential. We are thus driven, by the exclusion of other possibilities, to believe that the source of carbon to the green plant must be the *carbon-dioxide* of the air. This gas always occurs, although only in relatively small quantities, in the atmosphere, and is never absent from the water of lakes, rivers, &c. Carbon-dioxide also occurs dissolved in the water of a nutritive solution, unless special precautions are taken for its exclusion, while in the soil it is generally present abundantly. It is impossible to affirm right off whether a land plant obtains its carbon-dioxide from the air by means of the leaves or from the soil by means of the root. Experiment alone can settle this question; and experiment clearly teaches us that *land plants* cannot grow at all when they are prevented from obtaining carbon-dioxide from the air; at all events the carbon-dioxide absorbed by means of their roots is insufficient. Again, it may be easily proved that *submerged* plants are able to absorb all the carbon-dioxide they require by means of the surface of their leaves from that naturally dissolved in the water.

The fundamental thesis, therefore, we have to prove with regard to the assimilation of carbon by autotrophic plants is this: *the carbon-dioxide is decomposed by the energy of sunlight acting on the chlorophyll bodies of the living cells; the carbon is united with the elements of water to form carbohydrates while the oxygen is given off and escapes from the plant.*

[Following PFEFFER (Phys. 1st and 2nd eds.) and WIESNER (Elemente der Botanik), the production of organic material as well as its further alteration into the complex constituents of the living cell, are described in this book under the term *assimilation*. Objection has been taken to this nomenclature in a review of this work in the Botanical Gazette (1904, 37, 390) (see also Bot. Centrbl. 76, 257). The reviewer points out, with justice, that beginners are liable to get into difficulties if the formation of sugar out of carbon-dioxide in autotrophic plants, and the further transformation of sugar in heterotrophic plants, are both described by the same name. Nevertheless, there appears to us no good reason for accepting the alternative suggestion, viz. to describe as *synthesis* or *syntax*, the assimilation of carbon and so distinguish it from assimilation proper, for how should we then designate nitrogen assimilation or assimilation of minerals? In these cases we have no distinguishable simple bodies, corresponding to the sugar, which are assimilated, but rather potassium nitrate, or even the free nitrogen of the air. No good reason can be adduced for treating carbon differently from nitrogen, and therefore we have retained the older terminology, though with full cognizance of the difficulties involved in so doing.]

In order to render intelligible this extremely important thesis in plant physiology it will be necessary for us to investigate closely (1) the decomposition of the carbon-dioxide; (2) the significance of chlorophyll; (3) the importance of sunlight in the process; (4) the nature of the resulting products.

The decomposition of carbon-dioxide shows itself most obviously in the giving off of oxygen. The peculiar characteristics of water plants furnish us with a method of demonstration of this phenomenon equally serviceable for lecture purposes and for laboratory work. Fix a branch of *Elodea Canadensis*, or a submerged leaf of *Potamogeton*, to a glass rod in the manner shown in Fig. 22, and arrange that the branch or leaf while under water is illuminated by sunlight or artificial light. Presently we shall see issuing in regular succession from the cut end a series of small air-bubbles. This stream of bubbles may be accounted for quite simply. If we assume that the plant has been in the dark for some time it will have accumulated in its intercellular spaces, in addition to oxygen and nitrogen, a certain amount of carbon-dioxide. If this last be decomposed, the oxygen formed, in accordance with the known characters of gases, will occupy the same space as the carbon-dioxide did ; and hence there would appear no reason why air should escape from the cut surface. This will occur when the disappearing carbon-dioxide is replaced by diffusion from without, thus producing an excess pressure in the intercellular spaces of the plant. This excess pressure remains constant so long as carbon-dioxide is present outside the plant and undergoes decomposition within it. If we arrange an apparatus with the object of collecting the air which streams from several submerged branches exposed simultaneously to light, e. g. by covering them with a test-tube filled with water, we shall be able to study the gas much more conveniently and accurately. That the gas consists largely of oxygen is proved by the fact that it causes a glowing splinter of wood to burst into flame, and more exact analysis establishes the fact that it never consists of pure oxygen, but always has a demonstrable admixture of nitrogen. This is only to be expected, since, if the intercellular spaces of the plants have been, owing to the decomposition of carbon-dioxide, rendered richer in oxygen than the surrounding water, nitrogen must pass into the intercellular spaces from the water. Further, each individual air-bubble in its passage through the water, and finally the whole amount of gas present, must receive additions of nitrogen by diffusion so long as the experiment continues. No doubt we could arrange an experiment in such a way that pure oxygen would be obtained, that is to say by removing the nitrogen originally held in solution by the water, taking care to replace the carbon-dioxide lost in the process, and by preventing the entrance of fresh supplies of nitrogen. The form of the first experiment suggests several criticisms ; for instance, the stream of air-bubbles, at least at first, might be due to expansion of the air in the intercellular spaces by heating, and later on, to some extent, by thermo-diffusion. Evidence, however, that the stream of air-bubbles is entirely dependent on the presence of *carbon-dioxide* in the water disposes of all these objections. The addition of a small quantity of lime-water and the consequent precipitation of the carbonic acid (F. Schwarz, 1881), or the use of freshly boiled water, is sufficient to bring the stream of air-bubbles at once to a standstill, without doing any damage to the plant.

Fig. 22. From Detmer's Practical Plant Physiology.

The method just described is not only qualitative but may be made quantitative as well, since the number of bubbles which come off in a unit of time from a given specimen forms a means of measuring the amount of carbon-dioxide decomposed. It is true we must not compare several specimens with each other off-hand, for a vigorously active branch may give off few but large air-bubbles, while a weakly assimilating one may furnish many small ones. The

size of the bubbles, however, depends chiefly on the extent of the cut surface at the base of the plant experimented on, although even then the size is not constant in one and the same specimen.

Although the bubble method is often of service in quantitative experiments owing to its great simplicity, still it is a method confined in practice to *aquatic plants*. Further, it is impossible with this method to obtain fundamental data of a quantitative kind when absolute, rather than relative values are required. For this purpose *eudiometric* experiments are necessary, the principle of which is to expose portions of plants, especially foliage leaves, to sunlight in an enclosed chamber filled with an atmosphere rich in carbon-dioxide, and to investigate what alterations take place in the composition of the air. Methods of analyses have been perfected in many ways since the time of BUNSEN, and have become extremely exact (unnecessarily so for our present purpose) though somewhat complicated; more recently, however, BONNIER and MANGIN have introduced a much improved, and, in its latest form, exceedingly convenient apparatus by means of which gas analysis may be carried out rapidly and without involving laborious reductions (AUBERT, 1891). [If POLLACCI's criticism be correct (Atti Istit. Pavia, 1905), BONNIER and MANGIN's apparatus is perfectly worthless !] The results of eudiometric experiments may be briefly summarized as follows :—The volume of gas remains practically constant while assimilation is going on, because for every volume of carbon-dioxide that disappears an approximately equal amount of oxygen is produced.

Since the giving off of *oxygen* is intimately bound up with the assimilation of carbon-dioxide, it follows that there are many other methods of demonstrating the assimilation of carbon over and above the two that have been referred to. Oxygen possesses many properties, some purely chemical, some physiological, which may be employed in determining its presence. The literature on the subject contains many references to its purely chemical properties. Thus BEIJERINCK (1890) has shown that reduced indigo-carmine becomes blue again owing to the activity of assimilating plants; HOPPE (1879) placed a plant of *Elodea* in a sealed glass tube containing a dilute solution of venous blood; owing to the using up of all the oxygen, the solution then gave the characteristic reaction of haemoglobin; but the substance at once changed into oxyhaemoglobin (easily recognizable by its spectrum) whenever the tube was brought into sunlight. These methods are useful ones for demonstration purposes, but they have been as little used as methods of investigation as the physiological reaction described by BEIJERINCK (1901), who showed that if luminous bacteria are brought in contact with green Algae they are phosphorescent, but only when the green cells are assimilating, and that in the absence of oxygen the luminosity ceases. Another physiological method applicable to research has been more often employed, viz. that described by W. ENGELMANN in numerous memoirs, and summarized finally, under the title of the 'Bacterium method', in 1894. It depends on the fact that many bacteria, e. g. *Bacterium termo*, are capable of exhibiting movements in the presence of minute traces of oxygen. If one inoculates a drop of water on a slide with a pure culture of this bacterium, and surrounds the edge of the cover glass with a ring of vaseline to keep out atmospheric oxygen, the bacteria at first exhibit active movements in the fluid; but gradually their power of movement becomes less and less, until, when all the oxygen dissolved in the water has been used up, they at length come to rest. If bubbles of air be enclosed under the cover glass, however, these become centres of attraction to the bacteria in consequence of the presence of oxygen there; the bacteria move towards the bubbles, collect in their neighbourhood, and continue to exhibit movement for some time, although motionless elsewhere. The phenomena described—and this is a point of importance—are entirely independent of light, for they behave in the same

way whether the slide be placed in the dark or in the light. If in such a preparation we replace the air-bubbles by specimens of a unicellular alga we find that if kept in the dark all the bacteria quickly come to rest; as soon as the preparation is illuminated, however, the cells of the alga give off oxygen, and the oxygen at once exerts an influence on all the bacteria that happen to be near the cells, causing them to rush towards them and to move actively in their immediate vicinity (Fig. 23, *I, II*). When the light is removed the bacteria again distribute themselves over the field and come to rest, while each successive illumination once more induces movement and crowding round the alga. This method owes its frequent use more especially to its very great sensitiveness, for the presence of the minutest traces of oxygen may be demonstrated by its means. One may vary the sensitiveness by employing other bacteria, e. g. *Spirillum*, as well as organisms or cells belonging to other groups (Infusoria, Flagellata, or spermatozooids of sea urchins) since many of these react, some only to larger, some to even smaller quantities of oxygen than *Bacterium termo*. On the other hand, the method has its drawbacks; it must in any case be used with caution, as we shall have occasion to see by and by.

By means, then, of the methods which have been described it is possible to prove that *green* parts of plants can assimilate carbon-dioxide in light, and this is one of the best established facts in plant physiology. Nor is it difficult to show that this power is confined to *green* organs. Every experiment with a fungus or with a root demonstrates at once absence of any decomposition of carbon-dioxide; while, on the other hand, the parts of the plant with the darkest green colour, the foliage leaves, have long been known to be the most active members in carbon-dioxide assimilation. It is true that carbon-dioxide assimilation has also been often observed in parts of the plant otherwise coloured, but more careful study has always shown that these parts contain green colouring matter, which is simply masked by other pigments. The carriers of the green colour are the chloroplasts, which are special organs of the cell, capable of increasing in number by division, and developing the green colour under certain conditions. Thus it is possible to prevent its formation by omitting iron from the nutritive supply (compare p. 85), and also, in the higher plants at least, by keeping the plant in the dark. In both these cases the chloroplast itself—the protoplasmic basis of the chromatophore—is formed, but the chlorophyll does not develop; the chloroplast remains either colourless or yellow. Further, PFEFFER (1881) has shown by the eudiometric method, and ZIMMERMANN (1893) has confirmed his results by the bacterium method, that parts of plants which have become chlorotic owing to the absence of iron are quite unable to decompose carbon-dioxide, and hence we must look upon the green pigment as a factor of the highest importance in assimilation, and all the more so because we find that etiolated plants grown in the dark cause no decomposition of carbon-dioxide when first placed in the light. Decomposition will, of course, take place *after a certain length of time*, since the green colour becomes rapidly developed in light. Recently, EWART (1897) has made certain observations on etiolated cells, and has shown that if they be not too old or too young, an evolution of oxygen from them may be demonstrated by the bacterium method, even before the slightest trace of green colour has been developed. This would appear to confirm an older research of ENGELMANN'S, but further investigations are required to determine whether the interpretation EWART puts on his observations is correct or not. Further, it must be noted that an attraction of bacteria does not take place on *every* occasion. Bacteria respond by mobility, for instance, to other substances besides oxygen (Lecture XLIII), and it is impossible to affirm that etiolated chloroplasts may not contain substances which have an attractive influence on such organisms. At present we may be permitted to ignore EWART'S statements, and say that only cells containing

chlorophyll are capable of assimilating carbon-dioxide. [MOLISCH (l.c.) finds that etiolated chloroplasts are quite inactive]

That it is not the entire chlorophyllaceous cell, but only the chloroplast in particular that is the agent in the decomposition of carbon-dioxide, may be proved by the following observations :—If one allows two small circles of light to fall on a *Spirogyra* cell (with an open chlorophyll band) surrounded by bacteria, so that one plays on the chloroplast and the other on the colourless cytoplasm, it will be seen that an active assembling of bacteria occurs only in the first field of light (Fig. 23, *III*). Again, it has often been observed that solitary chloroplasts isolated from cells are able for a long time to assimilate whilst colourless cytoplasm is quite incapable of doing so. [MOLISCH'S (1904, Bot. Ztg. 62, I, i) results should be compared in this relation. He was able to show, with the aid of luminous bacteria, that assimilation of carbon-dioxide could be carried on by individual chloroplasts taken from dried dead cells.] The disputed question (compare KNY, 1897, 1898; EWART, 1898) as to whether such functional chloroplasts must be still surrounded by a layer of protoplasm or not, is of less interest in this relation ; it is, however, clear that *in the long*

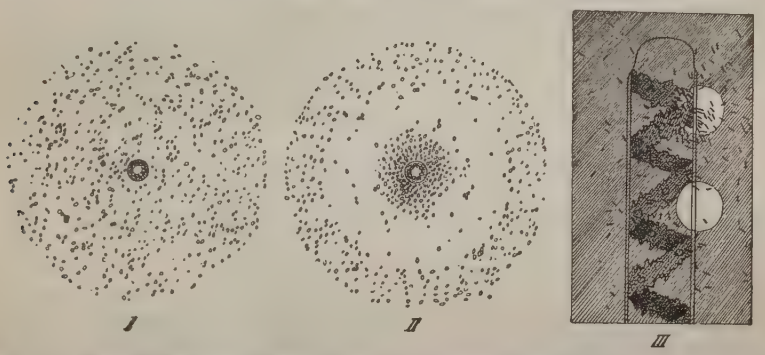


Fig. 23. *I*, a spherical, green alga centrally placed, and uniformly surrounded by bacteria (in darkness). × 150; *II*, the same preparation after a brief illumination; *III*, cell of *Spirogyra* illuminated at two places; the bacteria collect only where the beam of light touches the chloroplast. × 250. (After ENGELMANN, 1894.)

run it is only a chloroplast enclosed in protoplasm that can assimilate, and therefore it is not of so much importance to know how *quickly* it loses its capacity when isolated from the plasma. The dependence of the chloroplast on the protoplasm need not be directly connected with the process of assimilation.

Now that we have shown that it is only the chloroplast that assimilates in the cell, and that too only when it is green, we have next to attempt to settle the question as to whether it is the pigment itself, the chlorophyll, that is the agent in the decomposition of carbon-dioxide, or whether it can carry out its function in the absence of the protoplasmic basis.

This problem compels us to examine more closely into the physical and chemical characters of the chlorophyll. [The recent literature on the subject of chlorophyll is to be found in CZAPEK, I.]

In certain chloroplasts green granules or drops are visible, distinct from the colourless ground substance, and the green colour can be extracted by means of alcohol. This crude alcoholic solution of chlorophyll is characterized from a physical point of view by its fluorescence and by its absorption spectrum. By transmitted light the solution is a beautiful green, but deep red by reflected light, but this fluorescence appears only in the *solution* and never in the chloroplasts, and we should naturally conclude from this that the dye occurs in the chloroplast in a state of combination (REINKE, 1883). The spectrum of crude chlorophyll is shown at the top of Fig. 24.

It is characterized by the presence of six absorption bands, three of which are in the more refrangible (beyond *F*), the other three in the less refrangible part. The bands occurring on the other side of *F* appear distinct only when weak solutions are used; in strong solutions they merge into one. Of the three others, the first band ($\lambda = 670–635\ \mu\mu$) is by far the most intense; the second ($\lambda = 622–597\ \mu\mu$) and third ($\lambda = 587–565\ \mu\mu$) are much feebler. The figure further shows, just in front of line *E*, a band which belongs, not to chlorophyll itself, but to a decomposition product of it. A solitary chloroplast or a leaf presents an absorption-spectrum in all respects similar to that given by the solution, save that the bands as a whole are displaced somewhat nearer to the red end, whence we may conclude that the colouring matter in the chloroplasts is dissolved in a dense medium or occurs in a combined form.

The crude chlorophyll we have hitherto been studying is, however, by no means a simple body. As KRAUS (1872) has shown, it is possible, by shaking

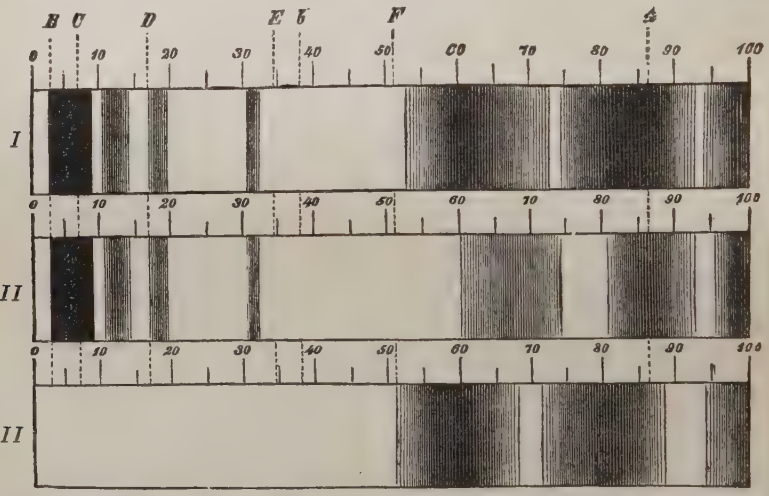


Fig. 24. Absorption-spectrum of chlorophyll (after KRAUS, 1872). *I*, alcoholic extract of the green leaf (crude chlorophyll); *II*, spectrum of the blue-green benzene extract; *III*, spectrum of the yellow pigment. (From DETMER'S Smaller Practical Physiology.)

up an alcoholic solution with benzene, to obtain a blue-green dye more soluble in the benzene, and a yellow dye which remains dissolved in the alcohol. The spectra of these dyes is shown in Fig. 24, *II* and *III*. Since the yellow dye has nothing to do with carbon-dioxide assimilation, we may dismiss it from our consideration in a sentence. It consists not of a simple compound but of a mixture of at least three bodies:

1. *Chrysophyll*, an undetermined hydrocarbon with formula $C_{26}H_{38}$, which can be crystallized and is closely related to, if not identical with, *carotin*, a substance widely distributed in the plant world. Its spectrum shows three absorption bands beyond *F*.

2. A substance which exhibits a spectrum with four absorption bands in the more refrangible part, which are, however, not identical with those of chrysophyll (*xanthophyll*, according to SCHUNK).

3. Another substance, which gives no absorption bands but produces entire obliteration of the violet and ultra-violet rays.

The colouring matter dissolved in the benzene is also by no means a simple body; it consists, in addition to true 'chlorophyll', of an admixture of a substance known as 'allochlorophyll', though in very small quantity. Great

progress has been made in the *chemical* investigation of true *chlorophyll* during the past few years, and research has shown that it is an exceedingly complicated body, in the composition of which lecithins and, perhaps, also proteid compounds take part. A detailed exposition of the chemistry of chlorophyll, from the pen of MARCHLEWSKI, will be found in ROSCOE and SCHORLEMMER'S Lehrbuch d. Chemie, vol. VIII, 1901. CZAPEK (1902) also gives a summary of the chemistry of chlorophyll; in both works, and especially in that of MARCHLEWSKI, a full list of the literature is given.

There is, further, a series of by-products of chlorophyll known to us, of which we may mention one only, viz. *phylloporphyrin*. This substance, occurring in the form of dark red-violet crystals, has a great likeness to a by-product of the colouring matter of the blood, *haematoporphyrin*. This likeness is demonstrated by comparison of the *absorption-spectra* of the two bodies. These spectra are identical save in one respect, viz. that the absorption bands of haematoporphyrin are displaced somewhat towards the red end (Fig. 25). A likeness is also apparent in the *chemical* composition of the substances, for haematoporphyrin has a composition represented by the formula $C_{16}H_{18}N_2O_3$, while that of phylloporphyrin is represented by $C_{16}H_{18}N_2O$. Both substances,

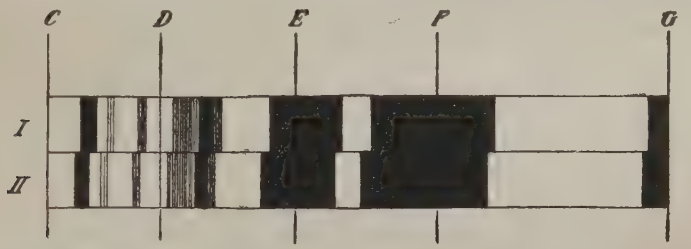


Fig. 25 Absorption spectra, *I*, of phylloporphyrin (in ether); *II*, of haematoporphyrin (in ether). After MARCHLEWSKI, in ROSCOE and SCHORLEMMER'S Lehrbuch der Chemie, vol. VIII. 1901.

on dry distillation, yield *pyrrol*. In spite of this agreement in chemical and physical characters, chlorophyll and the colouring matter of the blood have quite distinct *physiological duties* to perform.

After this digression on the characteristics of chlorophyll we may return to the consideration of the problem as to whether the dye alone, or only when in combination with the living protoplasmic basis of the chloroplast, is concerned in carbon-dioxide assimilation.

As a matter of fact the assertion has frequently been made that a solution of chlorophyll may, apart from the living substratum, be capable of abstracting oxygen from carbon-dioxide. KNY (1897) has, however, given most convincing proof that this is not so; for when he investigated oil-drops containing chlorophyll by the bacterium method, not a trace of oxygen was to be found. CZAPEK (1902) has carried this investigation a step further. He introduced chlorophylliferous oil-drops into colourless protoplasm, but was unable to find any evidence of the excretion of oxygen in the case of cells thus artificially provided with the dye. It would appear, therefore, that the plasmatic basis is quite as essential for the performance of the function carried out by the chlorophyll-apparatus as the dye, and that other selected parts of the cytoplasm cannot assume the duties of the plasma of the chloroplast.

Many facts, ascertained recently, of which we shall speak later on (Lecture XVII), render it not improbable that certain chemical compounds, perhaps quite independently of the living chloroplast, assist in the carrying out of the 'chlorophyll function'. The experiments of FRIEDEL (1901) and MACCHIATI (1903), aiming at the isolation of these bodies, have not, however, been successful

(HERTZOG, 1902). [BERNARD (1904. Beih. z. bot. Centrbl. 6) was unable to confirm FRIEDEL'S results, which must be considered as still requiring proof. Compare also MOLISCH, Bot. Ztg. 62, I, i.]

In the third place, the part played by sunlight in the process of assimilation must be emphasized. Each of the methods mentioned above shows in the clearest possible manner that the evolution of oxygen takes place *only in the presence of light and only in regions which are illuminated.* The bubble method demonstrates in the most self-evident way how assimilation ceases when a plant is gradually removed from the window to the back of a room, and how the process comes to a standstill even in light of relatively high intensity, which to our eyes would appear still sufficiently intense for the purpose. At the present moment we can only emphasize the fact that light is essential to the assimilation of carbon-dioxide, and postpone till later a consideration of the question of the quality and intensity of the light. We must first determine what is the exact *significance* of light in carbon-dioxide assimilation, and what substances are produced from the carbon-dioxide, or in other words, we must study the nature of the *first products of assimilation.*

The result of the gas-analysis method of investigation, viz. that the volumes of the carbon-dioxide operated upon and of the oxygen evolved are equal, provides us with a certain basis on which to work. The equality of the volumes suggests, for example, that carbon-dioxide is decomposed into carbon and oxygen. All experience, however, is contrary to the supposition that free carbon is produced; carbon never occurs as such in the plant, and it cannot be built up into organic material when it is artificially supplied in that form to the plant. All organic bodies contain in addition to carbon, at least hydrogen, and this can have been obtained only from the water which is present everywhere in the plant. If we now assume that hydrocarbons, which are the simplest organic substances, are those which originate in carbon-dioxide assimilation, then the oxygen must have been derived not from the carbon-dioxide alone but from the water also. If that be so, however, much more oxygen should be given off free than is, as a matter of fact, found to be the case. The experiments of BOUSSINGAULT (1868) moreover showed that hydrocarbons cannot be further worked up. On the other hand, the proportion observed to exist between the carbon absorbed and the oxygen given off agrees entirely with the manufacture of carbohydrate. Thus if we express the formation of starch or glucose schematically we obtain the following equations:—

$$\underset{\text{Carbon dioxide.}}{6CO_2} + \underset{\text{Water.}}{6H_2O} = \underset{\text{Glucose.}}{C_6H_{12}O_6} + \underset{\text{Oxygen.}}{6O_2}$$

or,

$$6CO_2 + 5H_2O = \underset{\text{Starch.}}{C_6H_{10}O_5} \quad 6O_2$$

In both cases the relation is $\frac{O_2}{CO_2} = 1$, as analysis itself also shows. The variations from unity are in many plants quite inconsiderable; thus BONNIER and MANGIN (1886) found it to be in the ivy, 1·08, and in the horse-chestnut and in *Syringa*, 1·06. These numbers tell us that a little more oxygen is formed than can be accounted for by the above formulae. This excess of oxygen reaches, however, a quite noticeable amount in other cases, for the same authors give the value of the fraction $\frac{O_2}{CO_2}$ in *Ilex* as 1·24. We shall take another opportunity of returning (Lecture XVI) to these cases, and consider, in our preliminary investigations into the origin of carbohydrates, the first-mentioned plants only. The development of carbohydrates as a result of carbon assimilation in green plants has, as a matter of fact, been established beyond all doubt.

Among the carbohydrates which result from the assimilation process,

starch has been known by far the longest, because it is the most striking and obvious constituent in the chloroplast. With the aid of a microscope we can often demonstrate its presence in the chloroplasts after a very brief exposure to light, if we take the precaution of seeing that the plastids were free from starch at the beginning of the experiment. We further find that when the plant has been kept for some time in the dark, not only is a further formation of starch prevented, but dissolution of what was present takes place. When the leaf, freed from starch and still attached to the stem, is placed in the sun early on a bright summer morning, we can, by using iodine solution, demonstrate the hourly increase in amount of starch. In the evening the chloroplasts are found to be so packed with starch grains that iodine solution turns the leaf perfectly black. In order to employ the iodine-proof to the best advantage it is advisable to extract the chlorophyll with hot alcohol, so that that pigment does not interfere with the colour reaction; for the proper demonstration of small quantities of starch it is preferable to treat the preparation either with chloralhydrate or with boiling water. When leaves of different plants are subjected to similar treatment with iodine, the resulting coloration gives us an approximate measure of the amount of starch formed, and it is easily seen that not all plants are able to produce starch to the same extent. A. MEYER (1885) has published a list from which one can see not only that variations occur in the capacity for producing starch, but also that their variations are characteristic of certain families. MEYER found :—

1. Very large quantities of starch in the Solanaceae and Papilionaceae.
2. Large quantities in the Papaveraceae, Crassulaceae, Geraniaceae, Oxalidaceae, Boraginaceae, Labiatae, Dioscoreaceae, and many others.
3. Moderate amounts in the Caryophyllaceae, Ranunculaceae, Coniferae, &c.
4. Small amounts in many Lobeliaceae.
5. Very little in many Gentianaceae and Iridaceae.
6. None at all in *Asclepias cornuti*; *Allium, Scilla* and many other Liliaceae, and in many Amaryllidaceae and Orchidaceae.

It is easily demonstrable, however, that even where no starch occurs there is still an active evolution of oxygen in sunlight, and that here also the volume of oxygen given off is equal to the amount of carbon-dioxide decomposed. It follows at once that *other carbohydrates* are also produced. More exact investigation further shows us that, even in the plants which are richest in starch, the starch is not the first product of assimilation. In the first place, it is highly improbable that an insoluble complex body like starch should arise at once, and in the second place, one finds even in favourable instances that the starch appears only at an appreciable interval after the commencement of the decomposition of carbon-dioxide. Thus KRAUS (1869) found the first visible traces of starch in *Spirogyra* five minutes after the illumination of the cells, and in other cases after a longer interval; but, by means of the bacterium method, it may be shown that the decomposition of the carbon-dioxide takes place *simultaneously* with illumination. The first products of assimilation must manifestly be soluble, and from these starch arises as a secondary result. The proof of this fact we owe to A. MEYER (1885), who has shown, by chemical analysis, that during assimilation large quantites of soluble carbohydrates are formed in plants without starch appearing, some reducing, some non-reducing, and further, that the same substances occur in plants which possess abundant starch. These results have been confirmed by A. F. W. SCHIMPER (1885), who draws the following conclusion from them, viz. that the difference between plants in the matter of starch formation does not consist in a difference in the activity of the assimilative process, but in the fact that while one series stores the soluble carbohydrates *as such* the other transforms them into *starch*. [According to A. MÜLLER (Jahrb. f. wiss. Bot. 1904, 40, 443), leaves con-

taining sugar construct considerably less solid than leaves containing starch.] In fact, it can be shown that many plants, normally without starch, can form it provided the sugar formed during assimilation be present in sufficient concentration. Such a concentration may be reached in many ways; by separating the leaves from the stem and so preventing the translocation of the manufactured carbohydrate, by providing an atmosphere rich in carbon-dioxide, and thereby occasioning an increase in assimilative activity, e.g. *Musa* and *Strelitzia* (GODLEWSKI, 1877), *Iris* (SCHIMPER, 1885), or by adding certain carbohydrates from extraneous sources. SCHIMPER (1885) for example, showed that *Iris germanica*, which is normally free from starch, can be made to form it if provided with a 20 per cent. solution of sugar. This method, varied in detail, has been used with great success by BÖHM (1883), and also by A. MEYER (1886), LAURENT (1887), and KLEBS (1888), to show that plants which have been freed from starch can form it from a sugar solution *in the dark*. Starch formation has thus nothing to do directly with the *assimilation of carbon-dioxide*; it occurs in *all* chromatophores, whether they contain chlorophyll or not, whether they are in light or darkness, and always occurs when soluble carbohydrate accumulates in large quantities in the cells. The degree of concentration of sugar from which the starch is formed varies in different plants. The authorities above named (and others) have further shown that, in addition to dextrose and levulose, which form the source of starch in so many plants, other carbohydrates (compare CZAPEK, 1902), such as mannose, galactose, saccharose, as well as alcohols such as glycerine, mannite, erythrite (at least in some plants), operate in the same way. The fact that *glycerine* may, in *very many plants*, be converted into starch, proves to us in the clearest possible way that we cannot argue backwards and say that all the substances employed in the formation of starch must arise during the decomposition of carbon-dioxide, for glycerine is apparently never formed as a product of assimilation.

We may, therefore, conclude that soluble carbohydrates are the first demonstrable products after the carbon of the carbon-dioxide had become united with water. This conclusion is, however, not satisfactory, especially from the chemist's point of view. The complicated nature of this substance is quite rightly emphasized, and we must, in consequence, search for a simpler body as the *first product of constructive metabolism*. There are many hypotheses forthcoming to aid us in our search for such a 'first product of assimilation'; of these we may refer here to one only which has proved of special significance in physiology, because it has led to a number of investigations. BAYER (1870) started from the similarity found to exist between the colouring matter of the blood and chlorophyll. Since carbon-monoxide was able to form a compound with haemoglobin he supposed that chlorophyll might show a similar capacity. Under the influence of sunlight he fancied the carbon-dioxide was broken down into carbon-monoxide and oxygen; the carbon-monoxide was united with water, forming, after evolution of more oxygen, formaldehyde. The following formulae express these chemical changes:—

$$CO_2 = CO + O; \quad CO + H_2O = CH_2O + O.$$

An optically inactive reducing sugar might then be simply produced from formaldehyde, i.e. formose, with the formula $C_6H_{12}O_6$ (BUTTLEROW, 1861; LOEW, 1886; compare also E. FISCHER, 1894). The fact that this sugar has not as yet been found in the plant does not appear to us to militate against BAYER'S hypothesis; but the criticism, that although carbon-monoxide, as JUST (1879) has shown, is not *injurious* to the plant as it is to the higher animals, it cannot undergo higher constructive metabolism, is more to the point. [Recently it has (CZAPEK, I, 428) been stated that carbon-monoxide may be assimilated even though it be a poison.] One may get over this difficulty easily by assuming

that formaldehyde arises from carbon-dioxide directly, without going through the intermediate product, carbon-monoxide. The direct origin is all the more plausible if we start, not from carbon-dioxide (CO_2), as we have been doing hitherto, but from carbonic acid (H_2CO_3). We are certainly entitled to do this, since chemists tell us that carbon-dioxide exists in aqueous solution only in the form of H_2CO_3; we know, further, that carbon-dioxide never occurs in the plant cells as a free gas, but always in the dissolved condition. The construction of formaldehyde would thus be represented by the equation :—

$$H_2CO_3 = CH_2O + O_2;$$
Carbonic acid = Aldehyde + Oxygen;

so that the hypothesis of ERLENMEYER (1877), which assumes formic acid as an intermediate product would be in accord with this view (compare CZAPEK, 1902) :—

$$H_2CO_3 = CH_2O_2 + O; \qquad CH_2O_2 = CH_2O + O.$$
Carbonic acid = Formic acid + Oxygen; Formic acid = Aldehyde + Oxygen.

Many arguments have been advanced from the physiological standpoint against the origin of formic acid and formaldehyde as intermediate products in carbon-dioxide assimilation; for example, the regular occurrence of these substances in the assimilating leaf has been doubted, and further it has been pointed out that formaldehyde is very poisonous to plants. More recently, POLLACCI (1900, 1902) has demonstrated with certainty the constant occurrence of formaldehyde in green leaves, while CURTIUS and REINKE (1897) observed the appearance of aldehyde in the green leaf in light and its disappearance in the dark, and especially noted (REINKE, 1899) that it occurred *not* as formaldehyde, but as a substance with the formula $C_8H_2O_2$. [POLLACCI also (Atti Istit. d. Pavia, 1904) has very strongly upheld the aldehyde hypothesis in a paper not long published, but a critical re examination of his work is still wanted (compare CZAPEK, I, 502–6).] In whatever way the question may be finally settled it would appear to us that the *deficiency* in a certain substance is a much better proof of its being an intermediate product in assimilation than its *plentiful occurrence*, since such intermediate stages would in all probability be passed through rapidly. It is important to remember in the discussion of this question that although formaldehyde, even in very small quantities (1 : 20,000), is intensely poisonous to plants, its poisonous effects could be avoided by its rapid construction into higher compounds. Further, it is extremely significant that, as yet, all attempts to make the chloroplasts use formaldehyde in the manufacture of starch have failed, and yet it must certainly take place if the hypothesis be correct. According to BOKORNY (1897), *Spirogyra* certainly can (although only in sunlight) *manufacture starch* out of sodium formaldehyde sulphite, and also from methylal. Since both these substances yield formaldehyde readily, BOKORNY concluded that the experiment furnishes evidence of the manufacture of starch from formaldehyde. Even if his opinion were well grounded, still BAYER'S hypothesis would not be *directly proved* thereby, for one could hold with equal justice that glycerine was the first product of assimilation (PFEFFER, Phys. I, p. 341), since starch is manufactured from glycerine, and that, too, in the dark. Experiments carried out by TREBOUX (1903) on *Elodea* show that this plant can live perfectly well in a 0·0005 per cent. solution of formaldehyde, but that it was quite unable to manufacture starch from it either in the dark or in the light.

The settlement of the question as to what is the first product of carbon-assimilation is not of great importance in *physiology*, and we will therefore confine our attention in the following pages to the consideration of the carbohydrates, which have been shown to be undoubtedly products of assimilation; and our knowledge of the phenomena taking place will be con-

siderably deepened if we proceed to investigate the subject *quantitatively* as well as *qualitatively*. Quantitative researches were first carried out by SACHS (1884), who was able, by using the iodine method, to obtain an approximate estimate of the amount of the assimilation products. His procedure was as follows :—He selected large, well-developed foliage leaves on plants growing in the open (*Helianthus*, *Cucurbita*, *Rheum*), and early in the morning cut out of one longitudinal half of the leaf (avoiding the larger veins) accurately measured portions from 50 to 100 sq. cm. area, in all about 500 sq. cm., and determined their dry weight. In the evening corresponding areas were taken from the other longitudinal half, which meanwhile remained attached to the plant and had been assimilating, and these were treated in the same way. In every case a marked increase in dry weight was observable, equivalent to the following amounts in grams per square *metre* per hour :—

Helianthus, 0·914 g ; *Cucurbita*, 0·680 g. ; *Rheum*, 0·652 g.

These numbers, however, must, for two reasons, be considered as representing only a fraction of the products actually manufactured. In the first place we know that every part of the plant suffers considerable loss of organic substance during carbon-dioxide assimilation, owing to respiration acting in the reverse direction (Lecture XVI), and, further, that there is a continual withdrawal of soluble carbohydrate taking place (Lecture XIV) into the stem. SACHS did not calculate exactly the loss from respiration, and disregarded it, beyond estimating it at about 1 g. per sq. metre during fifteen hours of assimilative activity. On the other hand, he instituted special experiments to estimate the amount of the *translocated* materials. With this object he investigated the loss in weight taking place during ten hours of continuous darkness, employing the same 'half-leaf' method above described. The temperature is, however, higher by day, so that more material is removed by day than by night. Since SACHS did not assume a greater loss in weight during assimilation than at night, his numbers may be taken, at all events, as *not too great*. He attempted to determine in another way the loss due to *translocation*, viz. by using *isolated* leaves ; in this case, also, the numbers obtained were certainly too small because storage of the products of assimilation must inhibit carbon-dioxide decomposition (Lecture X). The following numbers may be taken as giving only approximate minimum values under *very favourable* external conditions :—

Helianthus	per hour	and sq. m.	(Method 1)	1·882 g.	of dry weight	formed
,,	,,	,,	(,, 2)	1·7	,,	,,
Cucurbita	,,	,,	(,, 1)	1·502	,,	,,

Taking these numbers as a basis, SACHS reckoned that a vigorously active sunflower can manufacture 36 g., and a cucumber, 185 g. of dry weight during the course of a warm, bright summer day.

In SACHS's memoirs it is not the *dry weight* that is spoken of, but always *starch*, since at that time all products of assimilation were assumed to be deposited in that form. Ten years later, BROWN and MORRIS (1893) repeated SACHS's experiments, and confirmed them in all essentials, although they obtained *smaller* values than he did ; but they also showed that only a *fraction* of the products of assimilation occurred in the form of starch. In one case, for example, *Helianthus* showed an increase in weight of 8·566 g. per sq. metre in twelve hours (in addition to 4 g. translocated) but of this amount only 1·4 g. was starch, all the rest was sugar. Similar results are recorded in the works of A. MEYER and in several other memoirs which were published in the interval. MEYER'S studies on the *nature* of the sugars occurring in the leaf are therefore especially valuable, and for such investigations he found the leaves of *Tropaeolum* to be particularly adapted. Without discussing the methods employed we may give

the following results of his experiments in detail. The numbers indicate grams per cent. of the dried leaf substance.

	I.			II.		III.		IV.	
	a.	b.	c.	a.	b.	a.	b.	a.	b.
Starch	1·23	3·91	4·5	3·24	4·22	3·69	2·98	5·43	0·91
Cane sugar	4·65	8·85	3·86	4·94	8·02	9·98	3·49	7·33	3·35
Dextrose	0·97	1·20	0·00	0·81	0·00	0·00	0·58	0·00	1·34
Levulose	2·99	6·44	0·39	4·78	1·57	1·41	3·46	2·11	3·76
Maltose	1·18	0·69	5·30	1·21	3·62	2·25	1·86	2·71	1·28
Total amount of sugar .	9·79	17·18	9·55	11·74	13·21	13·64	9·39	12·15	9·73

Expt. I, a. Leaves analysed at 5 a.m.
I, b. Leaves cut off at 5 a.m.; examined after 12 hours of assimilation.
I, c. Leaves left on the plant and examined after 12 hours of assimilation.
Expt. II. Leaves left on the plant: (a) analysed at 9 a.m.; (b) at 4 p.m.
Expts. III and IV. Leaves cut off: (a) analysed in the forenoon; (b) 24 hours later—after they had been kept meanwhile in darkness.

These experiments are of the greatest interest, because they form the most detailed quantitative estimate as yet made and because they form a basis for further inquiry; their significance is not, however, so obvious. The carbohydrates, which occur in the leaf, group themselves obviously into two series possessing *quite distinct characters* :—

1. Those which increase in a marked degree during assimilation in light (I and II), viz. cane sugar, maltose, starch (di- and polysaccharides).

2. Those which increase in darkness (III and IV), viz. dextrose, levulose (monosaccharides).

This result, at first sight, appears somewhat surprising, since one would expect that the simpler sugars (dextrose and levulose) would appear first in the course of assimilation. Doubtless that is the case, but they become at once further altered and in consequence do not accumulate at all. The conversion takes place in at least three ways; in the first place these hexoses *migrate* out in very large quantities (compare I, c. with I, b.), next they become *respired*, and, lastly, from them *arise* cane sugar and starch. In addition to the construction of complicated carbohydrates a counter-formation of hexoses also takes place, and this preponderates in the dark (III and IV). If we assume that the dextrose and levulose are the *first products of assimilation*, then we must consider *cane sugar* and *starch* as *reserve substances*, which are temporarily stored by the assimilating cells, because the translocation of the assimilatory products does not take place so rapidly as their manufacture. Many of the features indicated in the tables are as yet inexplicable, and into the details of these we need not go; possibly, further research may afford an explanation of them. As to the relations subsisting between the di- and polysaccharides, BROWN and MORRIS have advanced the view that the starch arises from the cane sugar and the maltose from the starch. This view can scarcely be held as correct, and, moreover, it leaves unexplained many observed facts.

The assumption made by these authors that the *whole* of the carbon passes over, in the first instance, at once into *cane sugar* is, at least, not proved; doubtless this sugar is rapidly removed elsewhere. There is also the possibility that the carbon, during assimilation, is at once united with nitrogen and that the carbohydrate is formed by subsequent dissociation of this compound. A. MEYER (1885) held that the construction of proteids during the process of assimilation was not unlikely, but he was unable to support his view by any conclusive evidence. SAPOSCHNIKOFF (1895) has dealt with this question in greater detail. He determined the proportion of the dry substance formed during

assimilation which was carbohydrate in its nature, and found (1890) it to be, in round numbers (on an average of various experiments), 68 per cent., 87 per cent., and 64 per cent. He imagined that the remainder in each case, i. e. 32 per cent., 13 per cent., and 36 per cent., was *proteid*, and later on (1895) endeavoured to prove it. He also investigated the increase in carbohydrates and proteids during light in isolated leaves. An active construction of proteid could be determined especially when the leaves were immersed in nutritive solutions containing nitrogen. Further, a diminution in the light retarded the rate of formation of carbohydrates but relatively accelerated that of proteid. SAPOSCHNIKOFF, notwithstanding his anxiety to reach such a conclusion, was, however, unable, from these and other experiments, to establish the view that proteid was the 'first formed reserve of carbon-assimilation'; on the contrary, everything was in harmony with the conclusion that *proteid was a secondary product constructed from carbohydrates.*

The results which have been quoted as to the quantity of carbon assimilated are in accordance with those arrived at by the use of SACHS's half-leaf method or approximate closely to them. Although the errors in determining the area of the leaf surface be reduced to a minimum, still the inequalities in thickness of otherwise similar portions of the leaf would account for the inaccuracies in the amounts; for this reason one is limited to the use of a single leaf, because a comparison of surface areas taken from various leaves is bound to lead to errors. It is of interest therefore to draw attention to another method of determining the amount of the products of assimilation which we owe to KREUSSLER (1885–90), and which is certainly much the most accurate. He estimated how much carbon-dioxide was withdrawn by the leaf from the air, an amount quite independent of contingencies in the leaf structure. KREUSSLER investigated isolated twigs in appropriate bell-jars through which was made to pass a known quantity of air containing a known percentage of carbon-dioxide. He next determined how much of this carbon-dioxide passed out of the apparatus, and at the same time worked out how much carbon-dioxide was produced in the same period as a result of respiration. In this way the amount of carbon-dioxide actually absorbed by the plant could be accurately determined. Since KREUSSLER worked with air containing relatively high percentages of carbon-dioxide, and since the material experimented on was illuminated with electric light, his results furnish us with no information with reference to the amount of carbon-dioxide decomposed under *normal conditions of assimilation.* We need not enlarge on these results at the present moment, since they will have to be considered in another relation presently. BROWN (1899) has employed this method in a modified form. He worked with ordinary atmospheric air, whose percentage of carbon-dioxide was accurately determined, and further, employed ordinary daylight. BROWN also used in his bell-jars only single leaves, which, however, remained attached to the plant, so more easily guarding against withering than was possible with severed branches. Certain valuable data may be extracted from the preliminary account he has given of his remarkable experiments. A sunflower leaf, with a surface area of 617·5 sq. cm. absorbed in diffuse light 139·95 ccm. of CO_2 in five and a half hours, or 412 ccm. of carbon-dioxide per sq. m. per hour. Since one may reckon that 784 ccm. of carbon-dioxide on an average are required to construct one gram of carbohydrate it will be seen that, in the case cited, 0·55 gr. of carbohydrate was manufactured per sq. m., and this number corresponds pretty nearly with what BROWN found by weighing; the fact that it is considerably less than the amount found by SACHS is explained by the feebler intensity of light employed.

[In the preliminary account of their experiments, BROWN and ESCOMBE (1905, Proc. Roy. Soc. B. 76, 29) give only 0·4–0·5 gr. of carbohydrate per sq. m. per hour for *Helianthus annuus*. They draw attention to the difference between

their results and those of SACHS, and have found that the increase in weight in a leaf is *much greater* than one would expect from the amount of carbohydrate formed. They draw no conclusion from this (as one might well expect) as to the formation of compounds containing little carbon, but hold that SACHS's half-leaf method was a very inexact one.]

Since in SACHS's experiments a square metre of leaf surface of the sunflower produced 1·8 gr. of carbohydrate, it must have absorbed, roughly speaking, 1·5 litres of carbon-dioxide per hour. This would appear at first sight unlikely, taking into account the small amount of carbon-dioxide in the air as well as the capacity for absorption of the plant. We shall deal in our next lecture in detail with these relations.

Bibliography to Lecture IX.

AUBERT. 1891. Revue gén. de Bot. 3, 97.
BAYER. 1870. Ber. d. chem. Gesell. 1870, 68.
BEIJERINCK. 1890. Bot. Ztg. 48, 741.
BEIJERINCK. 1901. Kön. Akad. v. Wetensch. Amsterdam, 45.
BÖHM. 1883. Bot. Ztg. 41, 33.
BOKORNY. 1897. Biol. Cbl. 17, 1. (The older literature as to starch formation from various organic substances is given in this treatise.)
BONNIER and MANGIN. 1886. Annal. sc. nat., Bot. VII, 3, 1.
BOUSSINGAULT. 1868. Agronomie, 4, 300.
BROWN and MORRIS. 1893. Journal of the Chem. Soc. 63 (Transact.), 604.
BROWN. 1899. British Assoc. Address to the Chem. Section. Dover.
BUTLEROW. 1861. Compt. rend. 53, 145.
CURTIUS and REINKE. 1897. Ber. d. bot. Gesell. 15, 201.
CZAPEK. 1902. Ber. d. bot. Gesell. 20, (44).
ENGELMANN, W. 1894. Pflüger's Archiv, 57, 375.
ERLENMEYER. 1877. Ber. d. chem. Gesell. 10, 634.
EWART. 1897. Jour. Linn. Soc., Bot. 31, 554.
EWART. 1898. Bot. Centrbl. 75, 33.
FISCHER, E. 1894. Ber. d. chem. Gesell. 27, 3231.
FRIEDEL. 1901. Compt. rend. 132, 1131.
GODLEWSKI. 1877. Flora, 60, 218.
HERTZOG. 1902. Zeitschr. f. phys. Chem. 35, 459.
HOPPE. 1879. Zeitschr. f. physiol. Chem. 2, 425.
JUST. 1879. Wollny's Forschungen, 5, 79.
KLEBS. 1888. Unters. bot. Instit. Tübingen, 2, 489.
KNY. 1897. Ber. d. bot. Gesell. 15, 388.
KNY. 1898. Bot. Centrbl. 73, 426.
KRAUS. 1869. Jahrb. f. wiss. Bot. 12, 288.
KRAUS. 1872. Zur Kenntnis der Chlorophyllfarbstoffe. Stuttgart.
KREUSSLER. 1885–90. Landw. Jahrb. 14, 913; 16, 161; 16, 711; 19, 649.
LAURENT. 1887. Bull. Soc. bot. Belg. 26, 243.
LOEW. 1886. Journal f. prakt. Chemie, 33, 321; 34, 51 (comp. Bot. Ztg. 1886, 44, 849).
MACCHIATI. 1903. Revue gén. de Bot. 15, 20.
MEYER. 1885. Bot. Ztg. 43, 417.
MEYER. 1886. Ibid. 44, 81.
PFEFFER. 1881. Pflanzenphysiologie, 1st ed., 1, 185.
POLLACCI. 1900. Atti d. Istit. Bot. Pavia, N.S. 6, 45.
POLLACCI. 1902. Atti d. Istit. Bot. Pavia, 8.
REINKE. 1883. Ber. d. bot. Gesell. 1, 406.
REINKE and BRAUNMÜLLER. 1899. Ber. d. bot. Gesell. 17, 7.
SACHS. 1884. Arb. bot. Inst. Würzburg, 3, 1.
SAPOSCHNIKOFF. 1890. Ber. d. bot. Gesell. 8, 233.
SAPOSCHNIKOFF. 1895. Bot. Centrbl. 63, 246 (Review of the Russian paper published in 1894).
SCHIMPER, A. F. W. 1885. Bot. Ztg. 43, 737.
SCHWARZ, F. 1881. Unters. bot. Inst. Tübingen, 1, 97.
TREBOUX. 1903. Flora, 92, 49.
ZIMMERMANN. 1893. Beitr. z. Morph. u. Phys. 1, 29.

LECTURE X

THE ASSIMILATION OF CARBON BY AUTOTROPHIC PLANTS. II

Let us summarize briefly what we have learned up to the present on the subject of the assimilation of carbon :—the green plant decomposes the carbon-dioxide present in the air or water, evolves oxygen, and constructs carbohydrate. This process can take place only with the assistance of sunlight and in the chlorophyll corpuscles. We will endeavour in the present lecture to extend our knowledge of this phenomenon, more especially fixing our attention on the manner in which *external conditions* affect carbon assimilation in the plant, directly and indirectly. We shall take up the thread of our story where we left off in the preceding lecture and study first the amount of carbon-dioxide present in the air.

The amount of this gas present in the air has been very frequently and very accurately measured, and it has been found that it varies within very narrow limits, indeed it might be said to be a constant quantity so long as we avoid selecting for analysis the layers of the atmosphere immediately overlying the soil. Brown's (1899 [and 1905]) latest researches may be quoted in this relation, and he found on an average 2·8 parts of carbon-dioxide in 10,000 of air. This number agrees with the previous results recorded, e. g. by Reiset, who obtained 2·9, and those of the Montsouris Laboratory, where a number of independent observations gave, on an average, a proportion of 3 parts per 10,000. We will not enter into a discussion of the variations in the amount of carbon-dioxide in the air, for these are of no importance so far as the vitality of the plant is concerned. We have already referred (p. 103) to the question as to whether the carbon-dioxide of the air is necessary for the life of the green plant, and whether it cannot also draw upon the stores of carbon-dioxide present in the soil. That question was answered in the negative, still this is the proper place to refer again to the experiments which have been adduced in proof of this fact. It has been asserted by various investigators, most emphatically by Unger (1855), that the carbon-dioxide of the air was insufficient alone, and that that present in the soil must also form part of the supply. In opposition to this view, Moll (1877) was able to show that a plant which was prevented from obtaining carbon-dioxide save by its root, never succeeded in forming starch in its leaves, and manifestly suffered from want of the gas. It is also obvious that since the path from the root to the leaf is a long one, the transference must be slow, and that most of the carbon-dioxide will be abstracted on the way up by the chlorophyll bodies in the cortex of the stem.

If, however, the carbon-dioxide of the air be the source of carbon to the green plant, the question comes to be how is it possible that the amount in the air remains approximately constant, notwithstanding the fact that plant activity tends continually to diminish it. As a matter of fact, the amount of carbon-dioxide which the plant world abstracts from the air is very considerable, as the following statement will show. According to Sachs (1884), a sunflower with a leaf surface of about 1·5 sq. m. increases in dry weight to the extent of about 36 g. per diem (p. 114), and since 1·5 g. of carbon-dioxide in round numbers is produced from 1 g. of dry weight, it must extract about 50 g. from the air daily, or about 1·5 kg. in a month. If we assume the amount to be only 1 kg., taking into account possibilities of unfavourable weather, and imagine the whole surface of the earth covered with sunflowers, one to each square metre or one million to the square kilometre, then the sunflowers existing on the 135 millions of square kilometres of land surface would consume 135 billion kg. of carbon-dioxide in one month; and since, according to the usual estimate, there are 2,500 billion kg. of carbon-dioxide in the atmosphere, the supply

would last about twenty months. This is, perhaps, an exaggeration, since according to EBERMEYER'S (1885) reckoning, one hectare of forest uses up 11,000 kg. of CO_2, or one square kilometre uses up 1·1 million kg. in a *year*, whilst we have been making our estimates on the basis of sunflowers using 1 million kg. per *month*. This discrepancy, so far as our calculation is concerned, is of little consequence since all we want to demonstrate is that the supply of carbon-dioxide in the air is relatively small and must, in the course of a few months or years, be entirely used up by the activity of green plants. Since, as a matter of fact, no such decrease has, according to the most exact analyses, been observed, there must therefore be some processes taking place on the earth whereby carbon-dioxide is *produced* in quantity sufficient to replace what is used up.

As to the nature of these processes, on which the existence of organisms on the earth depends, a word or two of explanation must for the moment suffice. We are acquainted with several sources of carbon-dioxide. In inorganic nature volcanoes and springs bring up quantities of carbon-dioxide from day to day from the deeper layers of the earth's crust, and in the organic world the production of carbon-dioxide in animal respiration is a fact sufficiently well known. No estimate can, however, be made as to the total quantity so produced, for it is only in the case of the human organism that the necessary data are available. These data (PFEFFER, Physiol. I, 279), however, tell us that mankind produces daily 1,200 million kg. of carbon-dioxide, or 0·438 billions yearly, i. e. about $\frac{1}{6000}$ of the total amount in the air. Man, again, adds largely to this amount by the combustion of coal and wood, &c.; according to NOLL (1894, 166), Krupp's works alone give off daily into the atmosphere $2\frac{1}{2}$ million kg. of carbon in the form of CO_2. Then to this we must add the results of the respiratory activity of the plant world. Although we are unable to calculate the total amount of carbon-dioxide produced and used up over the earth, we may still conceive the possibility of a balance existing between them. Further, we can easily see how, owing to the continual movement of the air, a uniform distribution of carbon-dioxide in the atmosphere must be effected, so that one finds everywhere a percentage in round numbers of 0·03 per cent. present.

This all-important carbon-dioxide is not so uniformly distributed in water. It is well known that the absorption of a gas by water depends on its partial pressure and on temperature. Very different quantities will dissolve in water according as it absorbs the carbon-dioxide from the atmosphere or from the air in the soil. Further, the effect of temperature is so great that twice as much carbon-dioxide is absorbed at 0° C. as at 20° C. In addition to the amount of carbon-dioxide dissolved, chemically combined carbonic acid in the water is also available. Currents of water bring about rapid readjustment of the differences in the amount of carbon-dioxide present in different places.

Experience teaches us that the extremely small quantity of carbon-dioxide occurring in nature is no impediment to active assimilation in, and healthy life of, plants. Experiment, however, has also shown that an increase of carbon-dioxide in the air is accompanied by an increase in the products of assimilation. It has already been pointed out that there are plants which, although unable to manufacture starch in an ordinary atmosphere, can do so in one richer in carbon-dioxide, and that the occurrence of starch in them is the result of an increase in the amount of sugar constructed. We owe our knowledge of these facts to GODLEWSKI'S (1873) and KREUSSLER'S elaborate experiments. If we represent the normal amount of carbon-dioxide in the air by 1, and the amount of assimilation carried out under these circumstances by 100, then according to KREUSSLER :—

Proportion of carbon-dioxide .	1	2	3·5	7	17	35	220	440
Amount of assimilation . .	100	127	185	196	209	237	230	266 (?).

It will be observed that the rate of assimilation increases markedly from the very commencement, and that when the percentage of carbon-dioxide is thirty-five times the normal (i.e. about 1 per cent.) an optimum condition is reached. GODLEWSKI's (1873) figures, frequently at all events, show more strikingly than those of KREUSSLER (whose last figure is doubtful) an obvious decrease after the optimum is reached. The optimum, however, is much higher in GODLEWSKI's results than in those of KREUSSLER.

Glyceria spectabilis (Expt. 15).		*Nerium* (Expt. 24).	
Carbon-dioxide in air.	Carbon-dioxide decomposed.	Carbon-dioxide in air.	Carbon-dioxide decomposed.
3·1 %	2·10	3·6 %	4·31
7·0 %	4·73	13·2 %	3·62
10·4 %	5·75	18·5 %	3·23
13·9 %	2·27	28·2 %	2·42

[A research by PANTANELLI (1904) explains to some extent why different investigators have arrived at such varied optima for the proportion of carbon-dioxide present in the air. PANTANELLI shows that it really varies with the intensity of light; it is not apparent, however, why 0·1 per cent. of carbon-dioxide in the air should be so injurious to plants, as had been affirmed to be the case by BROWN and ESCOMBE (1902).]

It is not surprising that greater concentrations of carbon-dioxide should have an unfavourable influence on assimilation, since *all* vital processes are inhibited by that gas (LOPRIORE, 1895).

Let us turn now to the other question suggested at the end of the previous lecture, viz. how does the carbon-dioxide reach the assimilating cells of the leaf? Owing to the different external conditions under which aquatic plants live, the method adopted in their case cannot be the same as that existing in land plants. In the former case the carbon-dioxide dissolved in water must pass through the epidermal cells and may reach the other tissues either while still in the dissolved state, or it may escape as a free gas into the intercellular spaces, and by their means be distributed through the plant. The cuticle of aquatic plants presents no more impediment to the through passage of dissolved carbon-dioxide than it does to water, the medium of solution. The conditions are quite otherwise in the case of the cuticle of land plants, more especially that of the leaves. Carbon-dioxide gas is capable of penetrating such cuticles, even although they be quite impermeable to *water*, just as carbon-dioxide can diffuse through a layer of oil though water cannot. All the same, the partial pressure of carbon-dioxide in the atmosphere is so small that the amount which is able to pass through the cuticle is extraordinarily minute. Were there no other means of entrance available than by diffusion through the cuticle, practically no assimilation could take place under ordinary conditions. On the other hand, BOUSSINGAULT (1868) and BLACKMAN (1895) established the fact that formation of *starch* took place in such leaves as had obtained their necessary supplies only by way of the cuticle from air rich in carbon-dioxide.

It follows from this that in nature all highly organized leaves must be provided with carbon-dioxide in some other manner. The *stomata* at once suggest themselves as the portals for entrance of carbon-dioxide. Once the gas has reached the intercellular spaces by their means, it can easily penetrate into the individual cells after it has first become dissolved in the water of imbibition present in the cell-walls. The amount of carbon-assimilation will thus depend upon the number and distribution of the stomata and on the size of their openings. STAHL (1894) and MEISSNER (1894) have demonstrated this conclusively, for if a layer of vaseline be spread over leaves that bear stomata only on their undersides, any formation of starch in the leaves is prevented. At low temperatures leaves may be treated in this way without suffering any injury.

If we create a number of artificial stomata on the upper side of a leaf whose underside has been vaselined, by pricking it with a needle, or cutting it with a knife, or by removing altogether a strip of cuticle or the whole epidermis, the formation of starch takes place in the neighbourhood of such artificial apertures. It is easily understood why such starch manufacture is entirely local, seeing that the cells immediately underlying the gaps at once use up the small supply of carbon-dioxide in the air. MOLL'S (1877) experiments, which have been briefly referred to above, also show that starch formation is always limited to those regions in the leaf which have direct access to the carbon-dioxide, and it is on that account that the carbon-dioxide absorbed along with water from the soil is of no importance so far as the leaves are concerned.

The intercellular spaces, whose exits the stomata may be considered as constituting, are of the utmost importance for the transference of carbon-dioxide to the individual chlorophylliferous cells. Every one of these cells abuts somewhere directly on an intercellular space and is thus in continuity with the external air. Movement of carbon-dioxide in the intercellular spaces is effected, at least in the first instance, by diffusion. The entry into the leaf through the stoma depends on the partial pressure of carbon-dioxide in the air ; on reaching the assimilating cells the gas is completely absorbed and the pressure is reduced to nil, so that the necessary conditions for continuous diffusion are complied with. It cannot be doubted, however, that, in addition to diffusion, movements of the gases in the intercellular spaces in mass, resulting in a rapid supply of carbon-dioxide to the mesophyll, also take place. Variations in temperature as well as bending of the plant due to wind, resulting in changes in the shape of the intercellular spaces, must assist in producing such air currents.

Let us first of all consider stomata wide open and ask ourselves how it is possible for carbon-dioxide, though present in such a small proportion in the air, to enter the leaf through such minute pores in quantities so great that a sunflower is able to manufacture 1·8 g. of carbohydrate per square metre of surface per hour. A complete answer to this question is given in the recent researches of BROWN and ESCOMBE (1900). These authors started from a consideration of purely physical experiments. They allowed the carbon-dioxide of the air to diffuse into a vessel through a narrow opening in a thin diaphragm, placing a solution of potash at the bottom of the vessel. They found that the amount which diffused through was proportional, not to the *area* of the opening, but to its *diameter*. Thus, if an opening 4 mm. in diameter permitted two units of carbon-dioxide to pass through in a unit of time, one unit of the gas passed through an opening 2 mm. in diameter in the same time ; in other words, the amount of carbon-dioxide stood in the proportion of 2 to 1, while the surface extent of the aperture bore the relation of 4 to 1 ; hence as the size of the opening decreases the *rate of diffusion* must increase. When, therefore, numerous apertures are made in the diaphragm, and when the activities of these are added together cases must be conceivable where *diffusion takes place through a partition pierced by minute apertures as rapidly as if there were no wall there at all.* BROWN discovered that this result was reached if the individual openings lay so far distant from each other that they were unable to influence each other's activity, and that that was when their distance apart was at least ten times the diameter of the opening. When we apply the results arrived at from a consideration of such physical experiments to the inflow of carbon-dioxide into the leaf, the first thing to notice is that the stomata possess not a circular but an elliptical form. If we were now to consider the long diameter of the ellipse as the measurement on which diffusion directly depends it might be said that the *width* of the opening plays no part in the process, a view, as we shall see, directly opposed to observations. According to the detailed statements of STEPHAN the facts point to an entirely

different conclusion; an elliptical opening behaves exactly in the same way with regard to diffusion as a circular opening of the same area, each non-circular opening must be first of all reduced to a surface area of circular outline and the diameter of the latter taken as the figure on which diffusion depends. Thus BROWN and ESCOMBE estimated the effective size of the opening between the guard-cells of the stoma of *Helianthus* at 0·0000908 sq. mm., corresponding to a circular area of 0·0107 mm. in diameter. The distance of the stomata apart is equal to about eight times their diameter; they may thus interfere with each other, but only slightly. If we assume that the absorption of carbon-dioxide by the mesophyll is complete, we may conclude from the number of the stomata that 2·095 ccm. could be absorbed per sq. cm. every hour. As a matter of fact, the leaf absorbs only 0·134 ccm. per sq. cm., in order to construct a maximum of 1·8 g. of carbohydrate per sq. m., that is to say, only about 6 per cent. of the amount theoretically possible. From this it follows that the carbon-dioxide can only be absorbed slowly—for it must first pass through the cell-walls—so that the partial pressure a short distance below the stoma is far from being reduced to zero, while in physical experiments this condition is rapidly attained. 'The structure of a typical herbaceous leaf', says BROWN, 'is an admirable example of adaptation to natural laws, which in this particular instance have only recently become known to us. It illustrates in a striking manner all the physical properties of the multiperforate diaphragm, which, with its minute apertures, set at from six to eight diameters apart, and representing only 1 per cent. to 3 per cent. of free area, yet allows a perfectly free interchange of gases on its two sides, whilst at the same time affording every protection to the delicate structures underlying it.'

The stomata, however, are by no means always wide open, the size of the opening, on the contrary, varies with external conditions as we pointed out when discussing transpiration. Here, as on the previous occasion, we will content ourselves with referring to the two most important factors in the case, viz. light and atmospheric moisture. Since the maximum opening of stomata occurs in bright light we must recognize that as an adaptation of fundamental importance in the process of carbon assimilation. Increase of assimilation accompanies increase in light intensity (p. 125), provided that carbon-dioxide be present in sufficient quantity. An increase of atmospheric moisture brings about opening, a decrease a closing of the stomata, as has been already shown. Often long before a visible wilting of the leaf has taken place a complete closure of the stomatal slit is effected, as a result of the arrangement of the guard-cells already described. This closure of the stoma is essential to the preservation of life, otherwise withering must at once result; it is a safeguard against excessive transpiration, although at the same time a disadvantage so far as assimilation is concerned. It has been demonstrated by many experiments to how great an extent assimilation depends on the amount of water in the leaves; thus KREUSSLER (1885) observed that isolated branches, when brightly illuminated, showed a rapid decrease in the power of assimilation, while, according to NAGAMATZ (1887), withered leaves lose it altogether. In this case the closure of the slit is *alone* responsible for the deficiency in carbon-dioxide, and it must not be thought that assimilation depends on the existence of a certain degree of osmotic pressure in the interior of the cells. Assimilation is *not* lowered owing to loss of water in the case of plants such as mosses and lichens, which absorb carbon-dioxide by means of their cell-walls, so much as it is in the case of foliage leaves (BASTIT, 1891, 522; JUMELLE, 1892, 166), and in Algae assimilation has been observed to continue even after the beginning of plasmolysis (KLEBS, 1888). It must not, on the other hand, be affirmed that the amount of water present in the cell as such is a matter of no moment in the process of assimilation, for JUMELLE's researches show that even in lichens assimilation is undoubtedly dependent on the amount of water present.

We have thus learned that stomata constitute an apparatus of the very greatest importance for assimilative purposes, an apparatus whose functional activity is dependent in many respects on external factors. This dependence on the environment is still further increased by the fact that external factors affect the *development* of stomata. It is sufficient to note that stomata are not developed in darkness. The same is true of chlorophyll, whose first appearance and whose functional activity later is in the highest degree dependent on external conditions. Only in certain Algae (SCHIMPER, 1885) and in germinating Coniferae (BURGERSTEIN, 1900) is chlorophyll known to develop in darkness, but in all higher plants the chromatophores develop yellow colouring matter only, which has no effect on carbon-dioxide. Moreover chlorophyll decomposes in darkness, sometimes slowly, at other times quickly, in a word, both the origin and continued existence of chlorophyll depend on light. Further, a short exposure to light of feeble intensity is sufficient to induce a development of the green colour in the chloroplast, although subsequently placed in darkness. Again, greening is induced with varying rapidity, although, by all visible rays of the spectrum, and not by light of any definite wave length (REINKE, 1893). The temperature necessary for the formation of chlorophyll must not be too low; between 0° and 5° C., light is able to induce an increase only in the yellow colouring-matter already present (ELFVING, 1880), and even seedlings of Coniferae become green in darkness, as a general rule, only if the temperature be over 9° C.

Even when the chloroplasts develop normally, they cease acting as assimilative agents under conditions which scarcely affect many of the other functions of the cell, and which, at all events, act injuriously on life only after prolonged application. Thus the activity of the chloroplasts may be inhibited by extremes of temperature, by high percentages of carbon-dioxide, by anaesthetics and antipyretics, acids and alkalis [compare PANTANELLI, 1904], and also by prolonged insolation or accumulation of the products of assimilation (EWART, 1896). [According to ARNO MÜLLER (1904), the accumulation of the products of assimilation in leaves containing *sugar* retards the decomposition of carbon-dioxide.] At the same time respiration continues quietly, the chloroplasts appear unchanged and regain their powers of assimilation some time after normal conditions are reinstated. The fact that the *chlorophyll* persists unaltered during these interferences with its functions, shows that it is the protoplasmic framework that is affected. Both the component parts of the chloroplast must work in harmony if it is to carry out its normal functions (compare p. 109). Temporary inactivity of the chloroplasts may be unintentionally induced in the course of experiment—for example, it may take place very easily in amputated leaves owing to the accumulation of assimilation products—and in consequence, experiments with isolated leaves do not furnish us with accurate estimates of the amount of carbohydrates arising in the normal leaf (compare p. 114).

We have as yet spoken only of certain external factors which because they influence the stomata and chlorophyll, either as regards their structure or functional activity, are also of importance in the assimilation process. We must now consider external factors in so far as they affect assimilation *directly*, but it is obvious that no hard and fast line can be drawn between such external conditions as influence this process directly and those which influence it indirectly. One and the same factor may act in both ways. In fact, *carbon-dioxide* can, for example, when in greater concentration, inhibit the action of the chlorophyll, and thus can induce a decrease in assimilative activity, instead of causing the increase which we would expect on physical grounds. Since we have already treated of the influence of carbon-dioxide on assimilation, more especially in relation to its concentration, we have only to add here that

it cannot be replaced by any other compound of carbon, least of all by carbon-monoxide.

Along with carbon-dioxide we must study the *oxygen*. There are many facts which prove to us that, at the commencement of assimilation, no traces of oxygen are observable, and this is the more remarkable inasmuch as practically all the vital processes in the green plant are dependent on the presence of this gas. In the experiments on assimilation referred to above (p. 105) it was noted that free oxygen was definitely absent in reduced haemoglobin, and yet that the decomposition of carbon-dioxide could commence in such a medium; when decomposition does commence the experiment comes to an end, since oxygen is at once produced. We learn from EWART'S (1897) investigations, however, that certain plant-colouring matters are capable of combining loosely with oxygen, and we are led to believe that this capacity is more widely distributed than is generally assumed, and that, in experiments such as those quoted, it is not free oxygen but oxygen in loose combination that is at the disposal of the plant. At all events the plant's power of bringing about assimilation *ceases* in the long run in absence of oxygen; we have still, however, to decide whether this takes place when the loosely attached oxygen is used up or when the chloroplasts become inactive.

Heat, which has such a fundamental influence on plant life in general, affects assimilation also to a great extent. The determination of the dependence of assimilation on temperature from a quantitative point of view is by no means easy, because, in addition to the construction of assimilation products, a destruction of these same bodies, owing to respiration, is always going on at the same time, and because this latter process is dependent on temperature in other ways than the former. The most accurate experiments in this relation are those of KREUSSLER (1890). His researches show first of all that a gradual increase in assimilation is concomitant with a rise of temperature; the maximum assimilative activity is obtained somewhere between 25° and 40° C., followed by a decrease as higher temperatures are reached. Since assimilation soon ceases at temperatures above 45° C., while respiration increases with a further rise of temperature to over 50° C., there must be a certain degree of temperature at which the two processes neutralize each other. The following table summarizes KREUSSLER'S (1890) chief results on this subject :—

	Ricinus.					*Prunus laurocerasus.*				
Temperature.	25°	40°	45°	50°	60°	25°	40°	45°	50°	60°
a. mg. of CO_2 formed per hour in the dark owing to respiration . . .	8·5	15·0	14·8	16·4	0·75	7·7	19·9	21·7	21·3	2·5
b. mg. of CO_2 decomposed per hour in light . .	35·9	29·0	16·1	—29·9	—	20·9	41·7	3·1	—33·7	—
c. $a+b$ = mg. of CO_2 assimilated per hour in light	44·4	44·0	30·9	—13·5	—	28·6	61·6	24·8	—12·4	—

Looking at line *c* it will be noticed that the energy of assimilation in *Ricinus* is still as great at 40° as at 25°; at 45° it has sensibly decreased, and must soon after reach zero. In the case of *Prunus* it is greater at 40° than at 25°, but falls rapidly with increase of temperature, soon becoming nil above 45°. At all events, at 50° in light, in both cases, only carbon-dioxide is formed.

Line *b* shows the net profit or loss of the gaseous exchange occurring in light; *Ricinus*, in spite of concomitant assimilation, shows a smaller gain in carbon at 40° than at 25°, while in *Prunus* the conditions are reversed; at 45° the gain in carbon in *Prunus* is reduced to a minimum, and, beyond that, naturally entirely ceases, indeed there is a loss instead.

KREUSSLER (1888) has also investigated the lower limits of temperature. He found feeble but still recognizable assimilation taking place in bramble

leaves at −2·4° C. A similar result was obtained by JUMELLE (1892) in the case of other plants at quite low temperatures (−30° to −40° C.). According to EWART (1896), however, such low temperatures must speedily cause the chloroplasts to become inactive. [The dependence of the decomposition of carbon-dioxide on temperature has been investigated very accurately by MATTHAEI (1904). This author finds that the minimum lies near −6°, the optimum about 37°, and the maximum about 43°; the rise to the optimum is very rapid, and the fall beyond that point still more sudden.]

Although we have left the consideration of *light* to the last, it is not because it is of little consequence; on the contrary, it is of the utmost importance, and may be best discussed after the other influential factors have been considered. The fact that assimilation is intimately dependent on the presence of light may be proved with perfect ease, and branches of *Elodea* lend themselves readily to such experimental treatment (p. 110). [Very often the method is employed of partially covering a foliage leaf with pieces of cardboard, when the necessity for light in carbon-dioxide decomposition is shown remarkably clearly by the non-formation of starch in the darkened regions. HAUG (Bot. Gaz. 1903) has recently advanced some objections to this mode of experiment.] The evolution of bubbles of gas which takes place with a certain rapidity when *Elodea* is placed in a bright window decreases markedly when we withdraw the plants to the back of the room, and ceases entirely before what we should term 'darkness' ensues. The facts already referred to have not, however, been strictly speaking completely substantiated. In each green cell, as we have already seen, carbon-dioxide is formed during respiration as well as decomposed during assimilation. Respiration, however, may be said to be quite independent of light, indeed it goes on with equal activity in feeble light as in direct sunlight. There must, therefore, be a certain intensity of light under which as much carbon-dioxide is decomposed in the process of assimilation as is formed in the process of respiration, and then, of course, no air-bubbles at all will escape from *Elodea*, and even the specially sensitive bacterium method fails to show the existence of any assimilation. It is only by quantitative chemical methods that assimilation can be proved to occur, provided the amount of respiration in the parts concerned is known. When the intensity of light is still further reduced assimilation manifests itself only through a diminution of the amount of respiration, and comes to an end entirely when respiration ceases to make itself apparent.

More exact estimates as to the minimum intensity of light which can still bring about decomposition of carbon-dioxide are not forthcoming, and it is to be expected that individual species will exhibit differences in this respect. It is only too well known that most plants do not thrive in a room, and this is due for the most part to the indifferent lighting of our houses. Since several plants, such as *Clivia* and *Aspidistra*, do thrive in rooms, one might conclude from that, that the minimum light intensity required is less in their case than in that of others; but it has been shown that in these, as in other shade-loving plants, there is an entirely different reason for their power of endurance of weak light, viz. their reduced respiratory activity, on account of which less organic material is lost and there is less necessity for reconstruction.

Further we are not accurately acquainted with the precise way in which assimilation is dependent on the intensity of light. All researches agree on one point, viz. that assimilation of carbon is approximately proportional in amount to the intensity of light; it is questionable, however, whether this is the rule with higher intensities. REINKE (1883) found that the amount of assimilation was the same whether the plant was illuminated by direct sunlight or whether a lens with a magnifying power of 60 was used. But, as PFEFFER has noted (Physiol. 1st ed. I, 209) it may be easily imagined that a further increase in assimilation, following on increase of light, is impossible

owing to the deficiency in carbon-dioxide. Carbon-dioxide may be present in sufficient quantity under ordinary circumstances to employ all the energy of sunlight, but when light is artificially increased the usual amount of carbon-dioxide would constitute a sub-optimum. Further investigations may furnish us with a graphic curve representing the relation existing between assimilation and light intensity, similar to those which express graphically so many physiological processes, and showing a descending curve with further increase in light intensity, after a maximum point has been reached. So far such descending curves as those obtained by REINKE have been established only for lights of high intensity, which might inhibit the assimilative activity by *injuring* the chloroplasts.

[PANTANELLI (1904) has shown that in *Elodea* the optimum decomposition of carbon-dioxide takes place (when the water contains a normal percentage of that gas) when the light intensity amounts to one-quarter of that of direct sunlight, but as the light intensity is increased assimilation decreases. Doubtless plants with varying requirements so far as light is concerned ('shade-plants' and 'light-plants') behave differently in this respect (compare WEISS, Compt. rend. 1903). Attention has already been drawn (p. 120) to the fact that PANTANELLI has shown that the optimum intensity of light is higher as the amount of carbon-dioxide in the environment increases.]

Sunlight is known to be a mixture of rays of different refrangibility, of different wave length and of different colour, and it was long ago a question whether all these rays act in the same way or not. On this question much has been written, but the results which have been arrived at are by no means in proportion to the labour expended on them, on account of the difficulties that had to be overcome—or more correctly, perhaps, that have yet to be overcome, for no final answer to the problem has even yet been arrived at. The first researches on the subject were those of DAUBENY (1836); this investigator grew plants behind coloured glass plates, and this method is still of great service for demonstration purposes, although to be rejected for exact research, since the light so obtained is by no means monochromatic. We are unable to make much further progress by using coloured solutions, since although the light rays so filtered are virtually monochromatic (LANDOLT, 1894), still they are necessarily very feeble and consequently of little use physiologically. Hence in all recent research of an exact character the use of coloured media has been quite subsidiary to that of light subdivided into its components by means of a *spectroscope*. DRAPER (1843) was the first to put the solar spectrum to this use, and PFEFFER (1872), REINKE (1884), ENGELMANN (1884), TIMIRIASEF (1885), and others have made use of the same method. It cannot be described, however, without qualification, as an exact method, since the way in which the spectrum is produced involves the much wider dispersion of the more refrangible rays, so that equal areas in different regions of the spectrum cannot be equally effective. On the other hand, in order to increase the intensity of the light the slit of the apparatus must be opened so widely that the spectrum becomes no longer a pure one. The first source of error may be avoided by producing a normal spectrum with the aid of a diffraction grating. Such experiments have not yet been carried out, though REINKE (1884) has devised an apparatus by which comparable observations may be made without the aid of a diffraction grating. The principle of this 'spectrophore' (Fig. 26) is as follows :—

Light from a heliostat is made to converge by means of a lens O, and to pass through the prism P, by which it is decomposed. The spectrum falls on the screen SS_1. By means of two strips of wood (DD_1) selected parts of the spectrum may be blotted out, and the remainder of the rays are combined by means of the lens S into a beam of light, which is made to play on the plant.

This beam remains always in the same place, no matter how much and what part of the spectrum is obliterated. If a scale be inserted at SS_1, we can measure on it the wave lengths by observing Fraunhofer's lines, and are able to compare exactly equal parts of the spectrum with each other, by taking light of wave length, $\lambda = 700$–$640\,\mu\mu$ in one experiment, $\lambda = 640$–$580\,\mu\mu$ in a second, and $\lambda = 580$–$520\,\mu\mu$ in a third. The apparatus was later (Bot. Ztg. 1885) considerably improved, but no further experimental results have been published as a result of the use of the improved form.

In using the spectrum several modifications are adopted in individual cases. The macroscopic spectrum may be employed with the gas-bubble method to carry out eudiometric researches or estimate the amount of starch formed; a microscopic spectrum may also be thrown on the slide and the behaviour of an alga may be studied by the bacterium method. Under certain circumstances, when bacteria are introduced beneath the cover glass, aggregation of these at certain regions of the spectrum gives an indication where the maximum activity occurs; more exact quantitative values may be obtained if one and the same object be examined successively with light of different colours, and each time, by lessening the slit of the spectroscope, a minimum light intensity may be obtained, at which movement of the bacteria may still be observed. Naturally the most effective light through the narrowest slit will still suffice for assimilation, and vice versa.

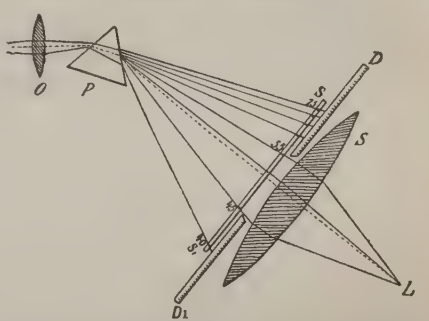


Fig. 26. Diagrammatic sketch of a spectrophore (after REINKE, Bot. Ztg., 1884, pl. I, fig. 2). *O*, projection lens; *P*, prism; *SS*, scale of $\lambda = 40$ to $\lambda = 75$; *D*, D_1, diaphragm for cutting off extraneous rays; *S*, converging lens; *L*, position of the plant experimented on.

A comparison of observations derived from *all* the researches which have been made brings out the following points :—

1. Only light of wave length between 770 $\mu\mu$ and 390 $\mu\mu$ is conducive to assimilating activity in green plants; these are approximately the same rays which are visible to us.

2. The assimilatory effect of different rays is unequal, but still not in such a way that some only are active whilst others lying beyond these are quite inactive.

If we express the wave length by abscissae and the activity of assimilation by ordinates, we obtain a curve which does not in the least correspond with the curve expressing the energy of sunlight obtained by LANGLEY.

On the other hand, no unanimity has been arrived at as yet as to the form of the curve, especially on the question as to whether there is *one* maximum or *two*, nor as to the exact position of the first and generally recognizable maximum point. This point lies in all cases somewhere in the red or yellow, the second maximum appears to lie in the blue region of the spectrum (compare the curves in Figs. 27 and 28). The importance of the second maximum is emphasized by ENGELMANN, who discovered it by the bacterium method. Since PFEFFER (Phys. I, 334, Fig. 53) could not convince himself of its existence by the use of the *same* method, and since the reasons which KOHL (1897) has brought forward more recently for its occurrence are by no means convincing, we must assume that the curve of assimilation has only one apex, somewhat as REINKE (Fig. 27) has described it, and that the maximum point lies without doubt in the less refrangible part of the spectrum. This conclusion, at once unexpected and important, was already drawn from a consideration of their own observations by the older investigators. Since the strongly refrangible rays are chiefly concerned in the decomposition of silver salts, e.g. in photography, it had been the custom to regard these as the chemically active ones. The data which have

been obtained with regard to carbon-dioxide assimilation, however, show that this generalization is inadmissible, and that red and yellow rays are also able to act energetically in a chemical manner.

These fundamental facts may be also demonstrated with the aid of light which has passed through bichromate of potash on the one hand and ammoniacal copper-oxide on the other. In yellow light photographic paper is only slowly blackened, but assimilation proceeds almost as rapidly under such illumination as in white light; on the other hand, light which has passed through ammoniacal oxide of copper exerts a vigorous decomposition in silver salts but has little or no effect in assimilation.

As to the position of the chief maximum point there is much dispute. REINKE considers it to be between Fraunhofer's lines *a* and *B* ($\lambda = 720$–$685\ \mu\mu$), ENGELMANN and TIMIRIASEF between *B* and *C* ($\lambda = 685$–$655\ \mu\mu$), PFEFFER (1871) between *C* and *D* ($\lambda = 655$–$590\ \mu\mu$). It ought to be easy to settle this question, though it is really of minor importance. In addition to the difficulties

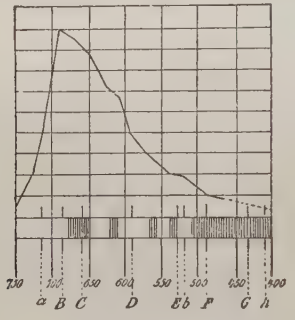


Fig. 27. Curve of evolution of gas-bubbles, compared with the absorption-spectrum of a living leaf. (After REINKE, Bot. Ztg. 1884, pl. I, fig. 6.)

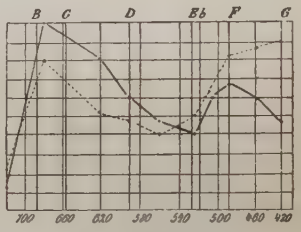


Fig. 28. Assimilation (firm line) and absorption (dotted line) in green cells between wave lengths $\lambda = 420$–750. (After ENGELMANN, Bot. Ztg. 1884, pl. II, fig. 1.)

which have been already alluded to (e. g. the attainment of a sufficiently *pure* spectrum with the necessary light intensity), one fact must be emphasized which ENGELMANN has drawn attention to and which PFEFFER has more recently (Physiol. 2nd ed. I. § 60) brought prominently forward. Chlorophyll has very different powers of absorbing light of different colours. The deepest absorption band of chlorophyll lies immediately in the vicinity of the assimilation maximum (Fig. 24), i. e. near *C* ($\lambda = 661$). Although this vigorously absorbed light has also the greatest effect in assimilation, still it can only be fully effective on structures of limited thickness. If we employ an ordinary leaf blade for experiment, the uppermost layers containing chlorophyll will absorb all the light of wave length about 660, and the layers lying below will be in darkness. Rays outside this limited region, e. g. those whose $\lambda = 630$, will be much less absorbed and will pass more deeply into the leaf, and will be able eventually to exert greater assimilatory activity than those which owing to absorption are rapidly altered. *Theoretically* the more interesting assimilating curve (*primary curve*) is naturally that investigated by ENGELMANN, not that *actually* observed on using thicker leaves. Even in layers of chlorophyll of limited thickness this primary curve is masked; the following values which ENGELMANN obtained from a comparison of the directly illuminated side and the reverse side of a cell of *Cladophora*, only 0·028 mm. thick, shows this clearly:—

FRAUNHOFER'S lines.	B–C.	D.	D½b.	E–B.	F.	F½G.
Assimilation (upper side) . . .	100	48·5	37·0	24·0	36·5	10·0
Assimilation (lower side) . . .	36·5	94·0	100·0	52·0	22·0	12·0

From these figures it is very evident that the maximum assimilation in parts of greater thickness tends towards the regions of lesser wave length.

We come now to the second question, why it is of such special interest to accurately determine the wave lengths of maximum efficiency. This is because it has been frequently assumed that a connexion exists between absorption and assimilation, that the maxima of absorption represented by the absorption bands must at the same time represent the maxima of assimilation. We have now to show that in the process of assimilation light *must be absorbed*, but it by no means follows that this light is completely transformed locally. Numerous colouring matters exhibit absorption spectra in the highest degree characteristic, without necessitating in consequence the postulating of a special function in the organism for the absorption bands these spectra exhibit. It is well to remember that the colouring matter of the blood has a very remarkable spectrum, but that it has *no significance on that account* in the animal economy. Further, in the case of silver iodide (OSTWALD, General Chemistry, II, 1070) a well-marked maximum light activity appears quite near line G, although at the same place, optically, absorption is quite feeble. The chief argument against the coincidence of absorption and assimilation is based on the physiological facts themselves, and it appears to us chiefly to lie in this, that attempts have always been made to establish a coincidence only between the position of the maximum of assimilation in the red and the absorption band near B–C, perhaps (ENGELMANN) also a corresponding coincidence in the blue, but it has never been possible to show any rise of the assimilation curve near the other absorption bands of chlorophyll. Let us examine ENGELMANN'S table (1884, p. 91), wherein *he* shows the connexion between assimilation and absorption, although it appears to *us* to support the opposite view.

Wave length ($\mu\mu$) .	718	680	622	589	558	522	506	486	468	431
Assimilation . . .	12·2	100·0	80·8	60·5	47·4	39·3	59·7	66·1	59·3	45·9
Absorption . . .	23·7	81·2	52·6	47·5	40·2	51·0	63·2	83·4	86·3	90·7

Notice especially the region $\lambda = 622$–522, where assimilation falls from 80 to about 40, while absorption decreases only a very little and has almost the same value at 522 as at 622; compare further $\lambda = 680$, where absorption = 81·2 and assimilation = 100, with $\lambda = 431$, where, in spite of *more vigorous* absorption, assimilation reaches a value of only 45·9.

If relations exist between absorption and assimilation these are of the *most complex* character and have still to be worked out. This is even more so in the case of the *yellow*, *red*, and *blue-green* cells of many Algae than of the *green* cells of the majority of plants. In regard to these Algae, ENGELMANN (1884) has made very interesting statements which, however, require confirmation by more extensive experimental evidence.

It has already been made obvious that the activity of light in the assimilation of carbon-dioxide must be bound up in a marked manner with light *absorption*. This may be assumed on theoretical grounds. The products of assimilation, starch or sugar, possesses a noticeable heat of combustion, while carbon-dioxide, from which they are constructed, being the end product, has no such value. The energy which the plant *gains* in the process of constructing organic substance *can* only originate from the *sun*, and hence, obviously, light, if it be altered into the chemical energy of starch, must disappear as *light* and an absorption of light must occur in the chlorophyll bodies. JULIUS ROB. MAYER, the discoverer of the Law of Conservation of Energy, took up a perfectly correct position with regard to the fundamental importance of this relation between plants and light when he wrote (1847, pp. 37, 38) :—'Nature has set herself the task of seizing the light pouring on the earth's surface and of storing this, the most mobile of all forces, in a fixed form. In order to gain this end she has covered the earth with organisms capable, so long as they are alive, of absorbing sunlight and of producing by its activity a continuous series of chemical

differentiations; these organisms are *plants*. The plant world forms a reservoir in which the fugitive solar rays are imprisoned, and are made to subserve certain uses.'

These reflections of MAYER have been completely confirmed, and we must recognize in *carbon-dioxide assimilation the source of the collective organic life of our globe, life which ultimately draws its energy from the sun.* Only *green* plants are able to fix the *sunlight* in this way, and all non-green parts of such plants as well as all non-green organisms are directly or indirectly dependent on this primary and most important synthesis of organic substance (compare Lect. XV).

Looked at from this point of view it is certainly of the highest importance to examine to what degree the green cells are able to make use of sunlight, how much of that energy they store and how much of it passes out unused.

It is possible to arrive at an answer to this question, in the first place, by theoretical calculation. Starting from BOUSSINGAULT'S work as a basis, PFEFFER (1871) assumed that a sq. m. of the leaf surface of *Nerium* formed starch at the rate of 0·000535 g. per second, and, estimating the heat of combustion of starch as 4,100 calories per g., concluded that 2·2 calories of solar energy is used per sq. m. per second, that is to say, less than 1 per cent. of the total solar energy (which is, according to POUILLET, about 333 calories per sq. m. per second). If, following SACHS, we reckon that a sq. m. of a leaf of *Helianthus* produces 1·8 g. of starch per sq. m. per hour, this would correspond in round numbers to 7,000 calories; but the amount of energy of the sun per sq. m. per hour is, according to POUILLET, equal to 1,200,000 calories, so that only about 0·6 per cent. of the total light energy is employed in assimilation. If we adopt AD. MAYER'S (1897) results we get a somewhat higher percentage, viz. 2·4 per cent., but MAYER made his calculations on the total solar energy for the *year*. Since a considerable portion of this solar energy falls on the earth's surface at a time when no vegetation is there, more than 2·4 per cent. of the actually available light would be effective. On the other hand, MAYER'S estimate of the total annual amount of solar energy at a quarter of a million calories must be too low, for LANGLEY'S results give double that number, so that after all corrections have been made the value would be again somewhat under 2·4 per cent. (compare also BROWN, 1899).

[According to the very careful researches of BROWN and ESCOMBE (1904), not quite 1 per cent. of the solar energy which falls on the leaf is employed in carbon-dioxide decomposition.]

Comparison of these calculations teaches us one thing at least, viz. that only a *small fraction of the total solar energy* is gained by the plant in assimilation; how large that fraction is, however, we cannot as yet exactly tell. DETLEFFSEN'S (1888) attempts to estimate this amount experimentally is therefore of the greatest interest. He made observations on the absorption of light in a leaf with the aid of a thermopile, placing the leaf alternately in air containing 10 per cent. of carbon-dioxide, and air free from that gas. In the former case, when assimilation was going on, he found that *more* light was absorbed than when there was no assimilation, and estimated that of the total amount of light 0·9 per cent. in one experiment, 0·3 per cent. in a second, and 1·1 per cent. in a third was absorbed. We must not place too much reliance on these numbers—for the sources of error are numerous and obvious—but DETLEFFSEN'S experiments appear to us to offer an interesting starting-point for further research, if aided by appropriate methods. It is quite possible that no more light is absorbed while assimilation is going on than when it is not, for the light which serves to bring about assimilation may, when assimilation is prevented, be transformed into heat, as in the case also of the not inconsiderable amount absorbed by the dead leaf and by a chlorophyll solution.

Why it is that decomposition of carbon-dioxide is possible only in the presence of *chlorophyll* we do not know. It has often been suggested that chloro-

phyll acts as a sensitizer. It is well known, for instance, that silver salts are affected only by rays of a certain wave length, and that red light, as every photographer knows, has no effect on them. By adding a colouring matter which absorbs red light, the silver salts become sensitive to red rays. The action of the colouring matters in this relation is by no means clearly understood, since not all dyes which absorb red rays act as sensitizers. Apart from that, there is one great distinction between these physical peculiarities and those presented by the chlorophyll body, viz. that silver salts are naturally affected by light, and their sensitivity is only increased by employing a sensitizer; the chlorophyll body, on the other hand, is unable to bring about the decomposition of carbon-dioxide in the absence of the green colour, so that we cannot look upon the dye merely as a sensitizer.

Seeing that the manufacture of organic material is necessarily bound up with the entry of energy into the plant, it may be asked whether this energy is of necessity always solar energy, and more especially that of the luminous rays. It might well be supposed that other forms of energy also, heat, electricity, and chemical energy, might be employed for the same purpose, and, as a matter of fact, it is very probable that some organisms can construct organic material out of inorganic with the aid of *chemical* energy. This mode of construction may be termed *chemosynthesis* in contrast with *photosynthesis*—the process we have already been studying (PFEFFER, Phys. 2nd ed.). We shall recur to chemosynthesis later on, but at the present moment, in view of the importance of 'photosynthesis', a few remarks as to the history of the subject will not be inappropriate. (Compare SACHS, 1875; PFEFFER, Physiol. 2nd ed. 1, 289; BROWN, 1899; [WIESNER, 1905].)

The essential preliminary basis on which this theory of carbon assimilation is built was laid down in a series of researches published in the last third of the eighteenth century. PRIESTLEY was aware that the atmosphere was rendered foul owing to the respiration of animals and to putrefaction and combustion, and he strove systematically to discover by what means nature counteracted this. In 1771 he established the fact that this duty was fulfilled by the *plant world*. It was *he* who in 1778 first made out that the air-bubbles escaping from partly submerged plants contained more oxygen than ordinary air. In the glass vessels which he employed in his researches he noticed, after long standing, the development of green masses which also gave off oxygen in sunlight, but since PRIESTLEY was unacquainted with the fact that these green masses were Algae, he thought he was observing a purely chemical process tending to the evolution of oxygen. PRIESTLEY does not seem to have clearly appreciated the importance of sunlight in the 'purification of the air', and it was INGENHOUSS who first drew attention to the fact that it was the green parts of plants that alone had the power of inducing this 'purification'. Both INGENHOUSS and PRIESTLEY were supporters of the phlogiston theory. SENEBIER was the first to investigate the subject from the standpoint of modern chemistry as founded by LAVOISIER, and his exposition of the subject appears to us to-day to be much more modern than those of his predecessors. He showed more especially the connexion between the carbon-dioxide and the evolution of oxygen, and was the first to make out that it was concerned in the process of the manufacture of organic material. It is true that, knowing how little carbon-dioxide was present in the air, he thought that plants absorbed the gas from the soil. TH. DE SAUSSURE (1804), however, first brought forward incontestable evidence that the *air* was the source whence the plant obtained its carbon-dioxide, and it was he who, by his exceedingly accurate experiments, placed our entire knowledge of the subject on a sound basis. Later on, his correct interpretation of the facts was neglected and 'humus' was once more thought to be of importance in the nourishment of the green plant, until, through LIEBIG'S sagacity and BOUS-

SINGAULT'S experimental work, SAUSSURE'S results obtained general recognition, and became what they are to this day, one of the foundation stones of plant physiology. The older investigators, for the most part, expressed no definite opinion as to the first product of carbon assimilation; later on, carbohydrates, amongst other things, were suggested, until SACHS announced that starch was 'the first visible product of assimilation'. We have already referred to the more recent developments in this relation, whereby it has been shown that the chloroplasts can construct starch out of carbon-dioxide but also from soluble carbohydrates as well; on these, naturally, all the non-green parts of the higher plants are dependent, as well as the great mass of Fungi, &c., which are entirely unable, since they possess no chlorophyll, to carry out carbon assimilation for themselves. Later on, we shall examine more closely the mode of nutrition of these heterotrophic organs and organisms.

Bibliography to Lecture X.

BASTIT. 1891. Revue gén. d. Bot. 3, 522.
BLACKMAN. 1895. Phil. Trans. R. Soc. B. 186, 504.
BOUSSINGAULT. 1868. Agronomie, 4, 375.
BROWN. 1899. British Assoc. Address to the Chem. Section. Dover.
BROWN AND ESCOMBE. 1900. Phil. Trans. Royal Soc. B. 193, 223.
[BROWN AND ESCOMBE. 1902. Proc. Royal Soc. 70, 397–412.
[BROWN AND WILSON. 1905. Proc. Royal Soc. 76, 29–137.]
BURGERSTEIN. 1900. Ber. d. bot. Gesell. 18, 168.
DAUBENY. 1836. Phil. Trans. p. 149.
DETLEFFSEN. 1888. Arb. bot. Inst. Würzburg, 3, 534.
DRAPER. 1843. Phil. Mag. 23, 161.
EBERMAYER. 1885. Sitzungsber. Münchener Akad. 15, 303.
ELFVING. 1880. Arb. bot. Inst. Würzburg, 2, 495.
ENGELMANN. 1882. Bot. Ztg. 40, 426.
ENGELMANN. 1884. Bot. Ztg. 42, 81.
EWART. 1896. Jour. Linn. Soc.; Botany, 31, 364.
EWART. 1897. Ibid. 31, 554.
GODLEWSKI. 1873. Arb. bot. Inst. Würzburg, 1, 343.
JUMELLE. 1892. Revue gén. de Bot. 4, 166.
KLEBS. 1888. Unters. aus. d. bot. Inst. Tübingen, 2, 489.
KOHL. 1897. Ber. d. bot. Gesell. 15, 111 and 361.
KREUSSLER. 1885–90. Landw. Jahrbücher, 1885: 14, 913; 1887: 16, 711; 1888: 17, 161; 1890: 19, 649.
LANDOLT. 1894. Ber. chem. Gesell. p. 2872.
LOPRIORE. 1895. Jahrb. f. wiss. Bot. 28, 531.
[MATTHAEI, G. 1904. Phil. Trans. R. Soc. B. 197, 47–105.]
MAYER, Ad. 1897. Versuchsstationen, 48, 67.
MAYER, R. 1845. Die organische Bewegung im Zusammenhang mit dem Stoffwechsel. Heilbronn.
MEISSNER. 1894. Beitr. z. Kenntn. d. Assimilationstätigkeit der Blätter. Diss. Bonn.
MOLL. 1877. Landw. Jahrbücher, 6, 327.
[MÜLLER, ARNO. 1904. Jahrb. f. wiss. Bot. 40, 443–498.]
NAGAMATZ. 1887. Arb. bot. Instit. Würzburg, 3, 389.
NOLL. 1894. In the Bonn textbook, 1st ed.
[PANTANELLI, E. 1904. Jahrb. f. wiss. Bot. 39, 167–228].
PFEFFER. 1871. Arb. bot. Inst. Würzburg, 1, 1.
PFEFFER. 1872. Bot. Ztg. 30, 425.
REINKE. 1883. Bot. Ztg. 41, 697.
REINKE. 1884. Bot. Ztg. 42, 1.
REINKE. 1893. Sitzungsber. Akad. Berlin, 527.
SACHS. 1884. Arb. bot. Inst. Würzburg, 3, 1.
SACHS. 1875. Gesch. der Bot. Münich.
SCHIMPER. 1885. Jahrb. f. wiss. Bot. 16, 1.
STAHL. 1894. Bot. Ztg. 52, 117.
TIMIRIASEF. 1885. Annal. Sc. nat. VII, 2, 99.
UNGER. 1885. Anatomie u. Physiol. d. Pflanze. Pest, Vienna, Leipzig.
[WIESNER, J. 1905. Jan Ingenhouss. Vienna.]

LECTURE XI

THE ASSIMILATION OF NITROGEN IN AUTOTROPHIC PLANTS

As the result of changes taking place in the carbohydrates which originate in the chloroplast, there arises a large number of important vegetable substances, of which only the materials of the cell-wall, fats, and the numerous organic acids may be alluded to here. All these substances are composed of the elements carbon, hydrogen, and oxygen only, but over and above there are numerous compounds in the plant which contain a fourth element, nitrogen; every plant, in fact, contains this element in a small but constant percentage (Lecture I, p. 5). The form in which nitrogen can be utilized varies in the different types, but we will confine ourselves at present to nitrogen requirements of the *green plant* of whose absorption of nutriment we have obtained so limited a conception. We certainly know far less about the mode of assimilation of nitrogen than we do of carbon, and this is the more to be regretted as nitrogen is an even more important food material than carbon. For protoplasm, the actual living substance, always contains nitrogen, while on the contrary those bodies which are composed of carbon, oxygen, and hydrogen only, cannot be considered as endowed with vitality.

Let us return to the water and sand-culture methods by means of which we were enabled to arrive at such definite conclusions as to the requirements of plants so far as the materials of the ash were concerned. When plants were grown in nutritive solutions we found that a very considerable increase in the plant's dry weight took place, but we also learned that such culture fluids must contain *all* the materials needful to the support of plant life (p. 81). The nitrogen supplied was in the form of a nitrate of calcium or potassium. We have now to settle the question as to whether such an addition is really necessary, whether the enormous quantity of free nitrogen, amounting to four-fifths of the atmosphere, may not be utilized by the plant. The answer to this question is emphatically in the negative; for although we may know of methods by which free nitrogen is brought into combination in inorganic nature, and although we shall, later on, find that there are certain plants (Lecture XIX) which are able to make use of free nitrogen, still we are compelled to deny this power to the ordinary green plant.

It is to BOUSSINGAULT (1860–61) that we owe the establishment of this view; he was certainly unaware of the special powers possessed by Leguminosae, although he carried out not a few researches on these plants as well. Since we purpose dealing with the problem of the absorption of nitrogen by the Leguminosae separately in Lecture XIX, we will confine ourselves here to plants not belonging to that group, and take as our example *Helianthus argophyllus*. BOUSSINGAULT performed three series of experiments on this plant; in the first series he grew the plants in pure sand without any addition of minerals, and especially with the omission of combined nitrogen; in the second series the sand had added to it the materials of the ash and potassium nitrate; in the third series materials of the ash and, in addition, potassium carbonate instead of potassium nitrate. The result of the research is summarized in the following table :—

	Dry substance; seed taken as = 1.	Organic substance formed.	Gain in carbon in 86 days.	Gain in nitrogen in 86 days.
A. (Sand) . . .	3·6	0·285 (g).	0·114 (g).	0·0023 (g).
B. (Sand, ash, nitrate)	198·3	21·111 ,,	8·444 ,,	0·1666 ,,
C. (Sand, ash, carbonate)	4·6	0·391 ,,	0·156 ,,	0·0027 ,,

We see from these numbers that in Series A and C nitrogen was almost entirely excluded; the limited gain in nitrogen amounting to about 2·3 mg. in

Series A, and to 2·7 mg. in C, being accounted for by the absorption of ammonia in the gaseous form from the air. Concomitantly with this exclusion of combined nitrogen there is a reduction in the amount of carbon and organic substance formed, as well as in dry weight generally. It is worthy of note, however, that an increase in dry weight may still occur, and that this increase is greater when plants are manured with the materials of the ash than if they be grown in pure sand. The amount of nitrogen present in the seed reaches a greater amount than can be accounted for by the limited supply in the ash.

Fig. 29. *Helianthus argophyllus*; 1, in the presence of potassium nitrate; 2, without it. (Proportionally reduced.) After BOUSSINGAULT, 1860, pl. 2.

The unequal development of plants treated in different ways comes out even more prominently from a study of BOUSSINGAULT'S figures than from a consideration of the data quoted above. At Fig. 29, two of BOUSSINGAULT'S figures have been reproduced, side by side, reduced to the same scale; 1, represents a plant from series B; 2, from series A, although it might stand equally well as a representative of series C, between which latter and series A the differences are not worth mentioning. From the figures it will be apparent that the normal plant may reach a height of 64–74 cm., and develop a prominent inflorescence, while that grown in absence of nitrogen reaches a height of only 11–14 cm., and produced a capitulum of very limited size.

This experiment shows with perfect clearness that *Helianthus* is unable to make any use of the atmospheric nitrogen. It also proves that potassium nitrate forms a very appropriate source of nitrogen for nutritive purposes, since the increase in dry weight in plants belonging to series B is nearly sixty times that of plants in series A. This great increase in dry weight is very surprising, when one remembers *how little* potassium nitrate the plants have been able to obtain. A pot containing 1½ kg. of sand received gradually in the course of three months 1·4 g. of saltpetre, and this amount was sufficient to enable two plants to reach their full normal development.

Many hundred culture experiments in water and sand have established the fact that nitric acid forms an excellent, not to say the best possible source of nitrogen for the great majority of plants. [How the divergent results arrived at by TREBOUX (1905) are to be explained it is, as yet, impossible to say.] In principle it is immaterial with what base the nitric acid is united, still, generally speaking, it is preferable to use such bases as are themselves essential, hence potassium or calcium nitrates, although they are more expensive than sodium nitrate, are most suitable in practice. It is impossible at present to say whether nitrites as well as nitrates play any part in providing nitrogenous nutriment to Phanerogams. According to MOLISCH'S (1887) researches these nitrites are very poisonous when present in high percentage, although in dilute solution (0·05 per cent. or less in the case of potassium nitrite) they are absorbed with avidity and undergo alteration in the plant; strange to say they are not oxidized into a nitrate, but, on the contrary, suffer reduction. Whether a green plant can or cannot pass through all stages of its development when supplied with nitrites only, is not known.

It is to BOUSSINGAULT that we owe our knowledge of the importance of nitric acid as a nutrient to the green plant; previous to his time it was believed, mainly owing to the influence of LIEBIG (1840), that ammonia was the chief source of nitrogen to the plant. This conclusion was readily arrived at because experience had shown that excellent results could be obtained by manuring with ammonia; it was not known that the ammonia in the soil is transformed into nitrate before it is absorbed by the plant. This nitrification (Lecture XVIII), due to the action of organisms in the soil, complicates the scientific explanation of the question as to the significance of ammonia in the nutrition of the green plant. The recent comprehensive researches of PITSCH (1887–1896) and of MAZÉ (1900) have conclusively proved that the nutritive value of ammonia must not be entirely denied; in the majority of green plants it is second only to nitric acid in value, inducing a definite development and considerable increase in dry weight. The fact that many plants thrive only moderately well when supplied with ammonia is accounted for by the fact that the ammonia salts when presented to the root in a more concentrated state produce an injurious effect. Carbonate of ammonia, on account of its alkaline reaction, is especially liable to cause injury to the plant, acting as a matter of fact like a poison. In the case of some plants, particularly maize and other Gramineae, ammonia is by no means of inferior value to nitric acid, for MAZÉ was able to obtain as great an increase in dry weight in maize, using at most a $\frac{1}{2}$ per cent. solution of ammonium sulphate, as when he supplied it with a solution of a nitrate. Similar results were obtained in cultures of *Brassica* and species of *Allium*. Forest trees also must be dependent on ammonia, since nitrates are seldom present in woodland soils. The significance of this is not so simple as it appears and will necessitate inquiry later on (Lecture XIX). So far as we know at present it is quite certain that in addition to plants which definitely prefer nitric acid (e. g. buckwheat, potatoes, turnips) there are others which get on just as well or even better with ammonia, so that it would appear to be a matter of indifference to such plants whether the ammonia is supplied to them in the form of a sulphate, a nitrate, or a phosphate; it is only the carbonate which, as already mentioned, is liable to produce injurious effects.

Let us now inquire into the sources of nitrates and ammonia in nature. Minerals which are of purely inorganic origin, and which at the same time contain nitrogen, occur only rarely in nature. ERDMANN (1896, Ber. Chem. Gesell. 29, 1710) obtained only very minute quantities (0·028 per cent. or less) of combined nitrogen in perfectly pure primitive rock. Nitrate of soda would at first sight appear to be an exception, but there is little doubt but that this form of nitrate occurs in nature as a product of organic activity [MUNTZ, 1889].

All evidence points to the fact that the total amount of fixed nitrogen available for plant nourishment nowadays arises from the chemical combination of free nitrogen gas. Processes are constantly taking place which result in the combination of gaseous nitrogen, but the converse process also occurs where free nitrogen arises from the decomposition of compounds. Every combination of nitrogen which is effected means a gain of nutrient to the typical green plant from a substance of no nutritive value, and every formation of nitrogen gas by decomposition of a nitrogenous compound means a loss to it. Hence these two processes as they occur in nature, and which we may briefly term *nitrogenous gain* and *nitrogenous loss*, are of special interest in relation to the question before us, and demand closer study on our part, though at the present moment we need not do more than briefly indicate the more important points in regard to the problem, reserving further details for study later on.

Nitrogenous gain takes place under various conditions. Apart altogether from conditions which may be created in a laboratory, there are only two methods of bringing about nitrogenous combination; one of these, in which ganisms play a prominent part, we will discuss later (Lecture XIX); the

other only need be referred to at the present juncture, viz. the oxidation of nitrogen into nitric and nitrous acids, which takes place under the influence of electric discharge in the atmosphere more especially in thunderstorms. Rain, mist, and snow carry this nitric acid in solution down to the soil in considerable quantities, as BOUSSINGAULT has clearly shown (1861, 325). The greatest quantity which he found in rain was 6 mg. of nitric acid per litre, but for the most part the amounts were 3, 2, or 1 mg., or even less. It cannot be said that any marked relation exists between the frequency of thunderstorms and the amount of nitric acid present in rain-water, for there is a relatively large quantity of combined nitrogen present in rain even at times when thunderstorms are entirely absent. It is possible that silent electric discharges, which are always taking place in the atmosphere, may account for this fixing of free nitrogen; perhaps, also, some of the nitric acid present in the air and carried down to the soil may have its origin in the dust which has arisen from the soil itself.

Altogether only a small amount of freshly combined nitrogen is added to the soil, not more than a kilogram per hectare per annum according to AD. MAYER (1901, 1, 205) [in tropical countries as much as 5 to 6 kg. (MARCANO and MUNTZ, 1889)], while the plants which grow on a surface of that extent, according to BOUSSINGAULT, use up about 50 kg. of nitrogen per annum. It is therefore essential that, in order that plants may continue to exist, the nitrogen derived from dead organisms should be returned to the soil to be used in the construction of subsequent generations. The nitrogenous compounds arising from dead animals and plants are decomposed by micro-organisms and, in general, changed into ammonia (Lecture XVII). This substance is greedily absorbed by the soil, and thus part, at least, of the ammonia arising from putrefaction will be fixed in the earth. Further, owing to the influence of micro-organisms the ammonia undergoes oxidation into nitrous and nitric acids (Nitrification, Lecture XVIII). In this way every soil contains in varying proportions nitrates, nitrites, and ammonia.

Losses of nitrogen in nature may, in the first place, be quite *local*. The *ammonia* arising from putrefactive decomposition—in so far as it remains unaltered—is only in part absorbed; a not inconsiderable amount passes off into the air in the gaseous form where it is oxidized into nitrous or nitric acids, or united with carbon-dioxide. This ammonia is returned to the soil in the course of atmospheric precipitation. According to AD. MAYER (1901, 1, 205), on an average about 2 kg. of nitrogen in the form of ammonia falls on a hectare of land annually, in addition to the single kg. of nitric and nitrous acids already referred to as brought down by rain. Volatile ammonia is *not entirely lost* to the plant; on the other hand, it may be transferred from one place to another; ultimately it may be removed out of reach of *land plants*, if it be carried by rain into the sea. The case is the same with *nitric acid*; when, owing to nitrification of ammonia, this substance arises in the soil it is then of service to the plants on the spot, only if it be at once absorbed by the roots. Since the soil cannot retain nitric acid, all of it not at once absorbed by the root will be washed away by rain and carried into rivers and, finally, into the sea. The development of nitrogen in the gaseous form is of far greater importance than the phenomena mentioned, which, on closer observation, resolve themselves into *translocations* and *transformations* of combined nitrogen, and not into actual *loss* of such. This evolution of free nitrogen takes place in the course of many decompositions (Lecture XVII), and also in certain combustion processes. If there were no organisms capable of making use of this free nitrogen, these losses would be irretrievable. As a matter of fact such organisms are well known to occur (Lecture XIX), and the power they possess is obviously of the greatest importance in the circulation of nitrogen.

We shall return to such vital phenomena later, meanwhile we may note

that a complete explanation of their mode of operation is not as yet possible. We may note only the fact that, despite the numerous researches which have been made during recent years as to the phenomenon of nitrogen fixation, we have no conception of the quantitative aspect of the question, so that it is quite impossible to say whether the fixation of nitrogen gas or its evolution is the more dominant feature in nature, or whether the one process is the exact balance of the other. When one remembers that originally no combined nitrogen existed on the earth, one is inclined to hold that the amount of combined nitrogen to-day is on the increase, and that as a consequence actually more organisms are able to exist now than thousands of years ago. Without doubt, the amount of living substance in nature depends on the amount of *nitrogen*, since nitrogen occurs only to a minimum extent in uncultivated soil.

Owing to the mode of occurrence of combined nitrogen the green plant can take it up in three different ways :—

1. It can absorb it in the form of ammonia or nitric acid *from the soil by means of the root.*

2. It can take up ammonia in the gaseous form *from the air by means of the leaves.*

3. It can absorb rain-water and nitrogenous substances dissolved in it also *by the leaves from the air.*

The *first* of these possibilities is really the only one we need consider. The power of leaves to absorb gaseous ammonia is undoubted (Schlössing, 1874); but the fact that this gas occurs in the air only in quite limited traces renders this capacity on the part of the leaves of no practical importance. On the other hand, large masses of manure may certainly appreciably add to the quantity of ammonia in the air, and it is quite possible that under these conditions it may exert an important influence on the development of many plants (Kerner, 1887). Any such favourable influence, it must be remembered, is only limited, since in higher states of concentration ammonia is very rapidly injurious. The absorption of *combined nitrogen* dissolved in rain through the leaves is undoubted, yet this amount also is so small that the ordinary land plant may be considered as entirely dependent on that absorbed from the soil. There is a large amount of literature dealing with the presence of ammonia in *uncultivated* soils, but into the discussion of these researches it is impossible to enter here. It will be sufficient if we refer to some of the results obtained by A. Baumann (1887) :—

One kg. of dry		mg. of nitrogen as ammonia.
Loam (derived from granite)	(Fir Mts. Bavaria)	22·27
Weathered gneiss	,,	11·05
,, porphyry	(Rhine Palatinate)	17·71
,, carboniferous sandstone		4·43
,, basalt	(Rhine Palatinate)	23·37
Loess without humus	(Münich)	6·58
Sandy soil	(Schrobenhausen)	2·23
Moorland soil	(Münich)	1·60

Soils which are *unworked* and *unmanured* vary greatly in the amount of ammonia which they contain ; basalt and loam soils contain the most, sandy and moorland soils the least. Further, the amount of ammonia decreases rapidly as the deeper layers of the soil are reached.

On investigating the amount of nitric acid in uncultivated soils the same author found it occurring for the most part in such minute traces that it was impossible to estimate it *quantitatively*. On the whole, then, the plant can obtain under natural conditions only a very small quantity of combined nitrogen in the soil, and its growth is thus dependent on the characteristics of the root-system already referred to, more especially the capacity it has

for drawing upon a large extent of soil for the absorption of a substance occurring but sparingly in it.

The continued growth of plants on natural soil indicates at least that they are always able to obtain the necessary nitrogen from it. It is otherwise with cultivated plants. Just as in the case of the materials of the ash, agriculture prospers according to the amount of nitrogen removed. If 50 kg. of nitrogen be extracted from a hectare at each harvest, and in large part removed permanently from the field, and at the same time not quite 3 kg. of nitrogen is added annually, the soil must become rapidly impoverished, and that condition can be remedied only by manuring. Part of the nitrogen removed may be replaced by the excrement of cattle, and this explains the favourable results obtained by manuring with excrement, customary even in the most primitive forms of agriculture. Excrement is insufficient of itself to replace the loss in nitrogen suffered by the land, for part of the nitrogen is sold off the land directly with the harvest or indirectly with the cattle ; the remainder, which is contained in dung, is entirely transformed into ammonia, and as such becomes further diminished by evaporation or is washed out after undergoing nitrification. The formation of free nitrogen in dunghills may be reckoned as a further source of nitrogenous loss. Thus, in all logical schemes of agriculture, artificial manuring with nitrogen is essential. Since nitrate of potash is too expensive, by far the most valuable manure is Chili-saltpetre (nitrate of soda), which occurs in immense beds in Peru, traceable in its origin to vital activity. This substance came into use in England seventy-five years ago and is still employed in very large quantities. In addition to Chili-saltpetre may be mentioned sulphate of ammonia, a by-product in the manufacture of coal gas and almost as valuable for the purpose as nitrate of soda. [Probably calcium cyanamide ($CaCN_2$) is also of great service as a nitrogenous manure.] Finally, those plants which bring about the fixation of the free nitrogen of the air are of the greatest importance in agriculture. These have been several times referred to but we shall speak of them in greater detail later.

Having now become acquainted with the compounds of nitrogen which may be made use of by the plant, and having noted that these substances are absorbed especially by the root, we may turn to the question as to where and how they are assimilated. As we remarked, however, at the beginning of the lecture, our knowledge of the assimilation of nitrogen is very defective. The final products of the assimilation of nitrogen at all events are proteids. These bodies are rightly considered as forming a series of chemical compounds of special importance, and hence deserve a few words at this stage in our work. Unfortunately, from one point of view, the advances in the chemistry of proteids, which have taken place during recent years, owing to the efforts of physiological chemists, have dealt rather with animal than vegetable proteids (compare the comprehensive expositions of HAMMARSTEN, 1895, COHNHEIM, 1900, KOSSEL, 1901, HOFMEISTER, 1902). Thus we do not even yet know whether the very important animal proteids occur in the plant kingdom also, while we know little or nothing as to the peculiarities of vegetable proteids. Consequently the following notes, which we extract from COHNHEIM'S works, must of necessity be very fragmentary. [Compare CZAPEK, Biochemie, vol. II.]

Proteids cannot be so easily represented by formulae as carbohydrates or fats. Five elements for the most part enter into their composition, hydrogen, nitrogen, oxygen, carbon, and sulphur, to which we may add also phosphorus. The relative amount of these elements in the different proteids varies greatly and little is to be deduced from the statements made as to the percentage composition of each. Generally speaking, however, proteids possess certain physical characters, give certain chemical reactions, and especially give rise to similar decomposition products, so that we may conclude that they form a natural series of compounds and not merely a heterogenous collection of organic bodies which cannot be catalogued under other and more fully studied groups.

From a physical standpoint the *colloidal* nature of proteids stands out pre-eminent. It is, doubtless, largely owing to the size of their molecules that proteids are *unable to diffuse* through parchment or animal membranes. Nevertheless they may be regarded as forming genuine *solutions* which have the peculiarity of being not very stable. Proteids coagulate on very slight provocation, and very often this coagulation is accompanied by considerable alterations in character. This coagulation is *permanent* and renewed solution is impossible without fundamental chemical change. Such coagulations are induced by alcohol, by boiling water, by strong mineral acids, as well as by certain so-called alkaloid reagents (phosphotungstic acid, tannic acid, &c.). On the other hand, proteids are transformed by salting out (especially by ammonium sulphate), into a solid and often crystalline condition without being chemically altered. This salted out proteid remains soluble.

The reagents mentioned above may be used as tests for proteids, but certain colour reactions may also be employed, of which the most important are the following :—

1. They give a blue-violet to red colour with caustic soda and a few drops of weak copper sulphate solution (*biuret test*).

2. Heating with concentrated nitric acid gives a yellow colour (*xantho-proteic reaction*).

3. Boiling with a solution of mercuric nitrate containing a trace of nitrous acid gives a rose to dark red colour (*Millon's reaction*).

4. Treatment with an alcoholic solution of α-napthol and concentrated sulphuric acid gives a violet colour (*Molisch's reaction*).

5. By boiling with caustic soda and a salt of lead a black precipitate is produced (*lead-sulphide reaction*).

Apart from the biuret test, the reactions described are effects produced, not by the proteid molecule *as a whole*, but by *constituent groupings* in it ; Millon's reagent, for example, acts on a different group in the proteid molecule than does the lead-sulphide test, and that again on a group not acted on by Molisch's reagent. One is thus able to differentiate in the proteid molecule a number of constituent groups, with which a study of the decomposition products of proteid has made us familiar. *Hydrolytic* decomposition more particularly has furnished us with especially valuable data, because obviously that method entails no very profound changes on the products of decomposition. Hydrolytic decomposition may be effected by boiling mineral acids as well as by enzymes (proteases ; compare Lectures XII and XIII) ; the products are similar in each case and we shall confine ourselves at present chiefly to the action of enzymes. By the action of proteolytic enzymes proteid is broken down, in the first instance, into smaller molecules, which still retain many of the characters of proteids ; there arise first the *albumoses*, which are no longer coagulable but may be precipitated by salting out. From these arise the *peptones*, which cannot be salted out, but which still respond to the biuret test. All subsequent decomposition products fail to show any biuret reaction, and thus are no longer proteid. Albumoses and peptones may still be considered as proteids, although many peptones contain no sulphur. Among the products of further decomposition we have next to recognize a sulphur-containing group. In what form this arises through the action of enzymes is not as yet fully understood ; cystin ($C_6H_{12}N_2S_2O_4$) rarely occurs in plants ; sulphates on the other hand, are apparently produced directly. [Intermediate substances between peptones and amino-acids have been discovered, coupled amino-acids or polypeptides, many of which have been synthetically prepared by E. Fischer (Czapek, Biochemie, II, 45).] Under the head of sulphur-free proteid groups we may recognize the following (Hofmeister, 1902) :—

I. *Belonging to the aliphatic series* :
1. Guanidin residue—$CNH.NH_2$.
2. Amino-acids.
a. Monamino-acids : leucin, glycocoll, alanin, aspartic, and glutaminic acids.
b. Diamino-acids : ornithin (united with guanidin to form arginin), lysin, histidin.
3. Carbohydrate groups.

II. *Belonging to the aromatic series* :
1. Tyrosin.
2. Phenylalanin.

III. *Heterocyclic groups* :
1. Pyrrol group.
2. Indol group.
3. Pyridin group.

From this general summary we may conclude that, among the proteid reactions, the xanthoproteic test and Millon's test for the tyrosin group, Molisch's test for carbohydrate, the lead-sulphide reaction for sulphur groups, are the most characteristic; the biuret test alone applies to the complete proteid molecule.

The classification of proteids given above is provisional ; it is based more on solubility, coagulability, &c., than on constitution. For our purpose the following summary will suffice :—

I. *True Proteids* :
1. *Albumins*. These bodies are soluble in pure water and can often be crystallized.
2. *Globulins*. Insoluble in water, soluble in dilute solutions of neutral salts, from which they may be precipitated unaltered by removal of the salts.
3. *Nucleo-albumins*. Distinguished by containing phosphorus.

II. *Proteids* ; compounds of albumin with other bodies, and more complicated than true proteids :
1. *Nucleo-proteid*. Compounds of proteid and nuclein ; occurring especially in the nucleus.
2. *Haemoglobins*. Compounds of proteid and haematin ; a decomposition product of haematin is haematoporphyrin (referred to at p. 109).

III. *Glutinoids*. Bodies of simpler composition than typical proteid, in which individual proteid groups are wanting.

So far as we know the true proteids occurring in plants belong especially to the globulins and nucleo-albumins ; albumins proper occur only occasionally. Owing to the *sparing* solubility of vegetable proteids, a fact which has been drawn attention to by WINTERSTEIN (1901), it has come about that many proteids in the plant have been quite overlooked, e. g. in *Vaucheria* (REINKE, 1883). We imagine that extraction with baryta water, 20 per cent. hydrochloric acid, &c., as tried by WINTERSTEIN, would result in the discovery of proteids in such cases.

Let us now return to our main problem, where and how are nitrates of potash and ammonia assimilated in the green plant ?

The nitric acid present in the soil obviously penetrates the protoplasm and is easily absorbed by the root in *dilute* solution. In many plants nitric acid occurs in such quantities that its determination presents no difficulty. Although micro-chemical methods fail to demonstrate its presence in other plants we must not assume its instantaneous alteration in the root-cells, since many secondary conditions may interfere with the ordinary tests (e. g. diphenylamin) for the presence of nitric acid. Tobacco, turnips, sunflowers,

potatoes, and wheat may be taken as examples of cultivated plants which contain large quantities of nitrates. In the last two the nitrate amounts to from 1·5 to 2·8 per cent. of the dry weight. Even greater quantities (15 per cent.) occur in *Amarantus*, to which may be added a whole series of weeds such as *Chenopodium*, *Urtica*, &c. The maximum of nitrate is found in the root, less in the stem and leaf, none at all in the seed. The nitrate increases as the flowering period approaches, and decreases when fruiting takes place. FRANK (1888) has shown that these plants contain nitrates only when they are able to absorb it by the root; if they be grown in nutritive solutions containing no nitrogen, or only ammonia, nitrates are entirely absent from them. Hence we may conclude that the nitrate is not formed in the plant, as BERTHELOT and ANDRÉ (1884) thought, but that it is absorbed from without and stored for future use. Such storing of nitrate, however, is by no means universal; many plants absorb no more than they absolutely require. The nitric acid is finally employed for the most part in the construction of proteid, and to this end the combination of nitrogenous and carbonaceous substances is especially necessary. We are accustomed to regard the carbohydrates as the material source of the carbon in proteid, but it can scarcely be doubted that other organic substances also, especially benzol derivatives, may serve this purpose. We are as yet quite ignorant as to what is the first product of union of the nitrogenous and carbonaceous substances. TREUB (1895) attempted to show that hydrocyanic acid was the first assimilation product in *Pangium edule*, but the proofs he has given in this case do not appear to us valid, and an extension of his hypothesis to other plants is scarcely justifiable. [TREUB (1905), in a more recent research, endeavours to show that hydrocyanic acid may be the first assimilation product of nitrogen-containing material. Numerous and interesting as the experimental data are which TREUB has brought forward, they are all in accord with the belief that the hydrocyanic acid is a *decomposition* product of metabolism.]

No definite answer can as yet be given to the question as to *where* the assimilation of nitrates and the construction of proteid takes place, though one is, generally speaking, inclined to hold the view that *all* plant cells may be seats of proteid synthesis. Many authorities hold that most of the proteid originates in the foliage leaves; SCHIMPER (1888, 1890), indeed, has expressed it as his opinion that nitrogen assimilation, like carbon assimilation, is dependent on chlorophyll and sunlight.

We may quote the following experiments in which he aimed at determining this point (1888). The leaves of *Pelargonium zonale* are known to contain generally an unusually large quantity of nitrate, and the amount present may be further increased by keeping the plant in the dark or in moderate light; it disappears, however, in strong light in a few days. Those parts of the leaf which contain no chlorophyll, such as occur in certain cultivated species of *Pelargonium*, exhibit no alteration of the nitrate they contain on exposure to light, and the same is true of the aerial roots of *Tradescantia selloi*. SCHIMPER also inquired into the origin of the large amounts of calcium oxalate to be found in illuminated leaves which were provided with calcium nitrate, and he supposed that the oxalic acid produced in the course of metabolism took the place of the nitric acid and united with the lime. (As to this supposed function of oxalic acid, compare BENECKE, Botan. Ztg. 1903; the subject will be again referred to in Lecture XVI.) There can scarcely be any doubt whatever that a vigorous synthesis of proteid takes place in leaves which are strongly illuminated, but the influence of *sunlight* and *chlorophyll* can only be indirect; their influence depends on the fact that carbohydrates are present during carbon assimilation in larger quantities in the region of origin than in other regions to which they must first be transferred; further, then, chemical construction may be more advantageously effected in carbon-assimilating cells

than elsewhere, and, finally, it is possible that when nitrates are present in a chlorophylliferous cell a part of the carbon assimilated may be employed directly in the manufacture of proteid without first going through the carbohydrate stage. Still SCHIMPER has *not* proved that a synthesis of proteid is *impossible* in the dark in those parts of the plant which have no chlorophyll; further, it has been often stated recently that nitrogen assimilation may take place in darkness. Thus ZALESKI (1900) has observed a vigorous synthesis of proteid in leaves of *Helianthus* which had been cultivated in KNOP's nutritive solution, when a large quantity (4 per cent.) of levulose was added at the same time; when no sugar was added a reduction in quantity of proteid formed was observed. SUZUKI (1898) conducted similar observations on barley, which he found to be able to construct proteid out of nitrates in darkness in the presence of glucose or cane sugar. Certainly researches are not wanting tending to contradict these results, and experimental treatment of the problem on an even wider basis is still desirable; but we may point to the analogy offered by many Fungi which assuredly form proteids in darkness out of nitrates, a fact which certainly does not militate against the existence of this capacity in Phanerogams. [GODLEWSKI (1903) has observed the formation of some organic nitrogenous compounds to take place in darkness from nitric acid; increase in proteid-nitrogen takes place only in light, a statement which is confirmed by LAURENT (1904).]

The absorption of ammonium salts in the undecomposed (nitrified) condition has been clearly proved to take place. Since it nowhere accumulates in the plant in appreciable quantity it follows that it must be rapidly used up. Moreover, its rapid transformation is *essential* on account of its poisonous properties. Ammonia is used up in the construction of proteid as well as in the formation of simpler nitrogenous bodies, of which we shall have to speak later on. The same question arises with regard to the synthesis of proteid from ammonia that we have left unsettled when speaking of nitric acid, viz. the influence of light. LAURENT (1896) held that light was essential [more recently (1904), LAURENT has expressed the opinion that assimilation of ammonia is also possible in the dark]; HANSTEEN (1899), on the contrary, observed that construction of proteid from ammonia took place also in the dark if the appropriate carbohydrates were present. He found that *glucose* was of great service in this respect but that *cane sugar* was useless; unfortunately he based his conclusions on microscopic investigations only. Here also comprehensive studies are urgently needed.

The problem as to the influence of light on the assimilation of nitric acid or ammonia is of the greatest importance for another and related reason. We have seen how light supplies the energy required in carbon assimilation to form out of carbon-dioxide chemical compounds containing greater supplies of energy. There can be no doubt that an expenditure of energy is also needed to bring about the synthesis of proteid from carbohydrates and nitric acid or ammonia, since, as AD. MAYER (1901, I, 174) has shown, reduction processes are certainly accompaniments of this synthesis. If it could be proved that the synthesis of proteid takes place only in the presence of sunlight, then we might assume that solar energy is the source of energy we are in search of. Since, however, this is not the case, we must look around for another form of energy, and we know of only one other form which we need consider, viz. *chemical* energy, set free whenever carbohydrates are oxidized. We will return to this subject in speaking of the phenomena of respiration, at present we need consider only one aspect of the process:—Synthesis of carbohydrates in the green plant is undoubtedly a case of photosynthesis; the sun provides the necessary energy for carrying this out; the synthesis of proteid, on the other hand, is to be regarded, at least in certain instances, as a case of *chemosynthesis*. It has already been shown that proteid synthesis is impossible in the absence of

carbohydrate synthesis, and that in the long run every case of proteid formation is dependent on sunlight, though indirectly.

Since the nitrogenous decomposition products which appear as a result of the breaking down of proteids, not only in the plant but apart from it, as a result of boiling in acids, are always those mentioned at p. 139, it may be assumed that the *synthesis of these bodies, which we term amides, precedes the synthesis of proteids* [perhaps with the formation of polypeptides as intermediate products]. In fact, these substances, more especially asparagin, are known to be of widespread occurrence in plants, although it has not as yet been shown whether they are, primarily, intermediate stages in the formation of proteid from ammonia or nitric acid, or secondary products resulting from the decomposition of already formed proteid. Although FRANK and OTTO (1890) found that leaves generally contain more asparagin in the evening after illumination than in the morning, we must not conclude on that account that synthesis of asparagin has taken place; it might just as easily arise from an increase in the proteid contents of the leaf and a concomitantly increased decomposition of proteid. It may be possible, however, to determine whether the amides found are produced there by the breaking down of complex molecules or by the synthesis out of simpler bodies. Perhaps an investigation of leaves whose proteid is prevented from escaping from them by their removal from the stem, may serve, in comparison with normal leaves, as a starting-point. A more comprehensive view of the process would be obtained if we could establish a vigorous assimilation of carbon and of nitrogen in leaves while preventing a concomitant production of proteid. *Sulphur* is present in proteids under all conditions, and although this element is required only in small quantity it might be still possible perhaps to obtain a more vigorous anabolism of amides in the leaf-blade by withdrawal of sulphates and finally to induce a subsequent combination of these into proteids by adding sulphur afterwards. Experiments in this direction are still much needed.

Under present circumstances it is of interest to know that the power of the plant to construct proteid out of the nitrogenous organic substances named above, and others also, has been repeatedly proved. Older experiments (for literature see PFEFFER, Phys. I, 397) have shown how substances like urea, glycocol, asparagin, leucin, tyrosin, guanin, creatin, hippuric acid, uric acid, &c., may be supplied to plants in water-cultures in place of ammonia and nitric acid, whilst more recently LUTZ (1899) has shown that, in addition to acetamide, methylamylamin, ethylamylamin, &c., may be employed. The plant can recoup itself so far as nitrogen is concerned from such substances, although they are not all equally good for the purpose. If a marked increase in dry weight takes place, proteid must have been synthesized from such nitrogenous bodies. It is a well-established fact, however, that the transition to proteid is never direct, but that it is usually preceded by decomposition processes. This is well known to be the case with hippuric acid, which breaks up into benzoic acid and glycocol, the latter only undergoing further transformation. Further, all these bodies are easily changed into ammonia through the action of micro-organisms. Although it has been often expressly stated that the formation of ammonia could not be demonstrated in certain experiments, it by no means follows that it did not occur. It may well be that the ammonia is at once absorbed by the plant as soon as it appears. The systematic exclusion of micro-organisms has not been considered worth while in the majority of the researches, and in those which have been carried out with antiseptic precautions (LUTZ, 1899), other sources of error are not absent (compare SCHULZE, 1902). Notwithstanding, we cannot doubt that a transformation of amides into proteids takes place in the plant. In Lectures XIII and XIV we shall learn that the plant produces such substances in the course of metabolism and how it reforms proteid from them. In this case, as in the older water-culture

experiments, the working up of amides takes place in light. We have still to inquire, however, whether this is possible in darkness, and HANSTEEN'S (1898) researches have shown that this is indeed the case.

HANSTEEN provided the plants he experimented on with carbohydrate and nitrogen, either by adding these bodies to the culture solutions (*Lemna minor*) or by injecting the solutions into the plant through a wound. It is difficult to see how it was possible to exclude micro-organisms in the first case. Moreover, unfortunately, he maintained his experiments for only a few days, so that it was impossible to arrive at any conclusion as to whether continued growth could go on under these conditions. In addition he employed exclusively microscopic methods for determining the presence of proteids, viz. by iodine or Millon's reagent. It is well known that such evidence, especially if *quantitative* in character, is extremely unreliable. Although HANSTEEN'S experiments cannot be considered as free from doubt, we must quote his results here for want of better. Perhaps EFFRONT'S (GREEN-WINDISCH, 1901, p. 166) statements, which tend to show that asparagin accelerates the action of diastase, are of importance in deciding as to HANSTEEN'S work (p. 151). [REINHARD and SUSCHKOFF (1905) have still further called in question HANSTEEN'S results.] HANSTEEN found :—that in the dark, proteid was produced from urea in the presence of cane sugar just as well as with glucose; that asparagin, glutamin (and also, as previously mentioned, ammonium compounds) formed proteid only in presence of glucose ; that proteid was formed from glycocoll only in presence of cane sugar ; that as a rule no proteid was formed from nitrates, leucin, alanin, creatin, together with the carbohydrates experimented on, although there are, doubtless, other carbohydrates whose presence may make such a transformation possible.

The chief result which HANSTEEN arrived at, viz. that in the dark proteid is synthesized from amides and carbohydrates, has been confirmed by MALINIAK (1900) by quantitative analysis. He observed, on supplying asparagin, that synthesis of proteid took place in the dark in maize seedlings which had been deprived of their endosperm, and also in etiolated leaves of *Faba*. The data adduced to prove these facts are by no means very convincing, and the experiments, as is often the case, were carried out on too miniature a scale. In opposition to HANSTEEN, MALINIAK found synthesis of proteid from asparagin taking place just as well in the presence of glucose as of cane sugar. It has been shown also that synthesis of proteid takes place in the dark in resting and also sprouting bulbs, tubers, and roots, without any absorption of nitrogen from without, and without any increase in nitrogen ; this has been demonstrated recently in a series of researches by ZALESKI (1901) and IWANOFF (1901 a) who employed exact methods of chemical analysis. Whence these proteids arose is not certain, but in every probability from amides. [In young seeds, also, ZALESKI (1905) has proved the synthesis of proteid from albumoses, amido-acids, amides, and organic bases in the *dark*.]

It may be seen from these remarks how little we really know on these problems, and how desirable it is that some one should produce a really 'classical' work on the subject, for it is impossible to give a complete picture of the process of nitrogen assimilation based on such literature as we have been hitherto considering. A complete exposure of the numerous contradictions occurring in the literature at present available cannot be undertaken at present and hence much research, which may afterwards turn out to be of the utmost importance, has not been referred to at all.

Just as in the case of the carbon and nitrogen, so also the materials of the ash are 'assimilated' in the plant; the majority of them, at least, are probably built up into organic compounds. Since, however, we are for the most part completely in the dark as to which elements of the ash are of service in the assimilation of organic substances, a discussion of the process of assimi-

lation of the majority of these minerals is as yet out of the question ; we may, therefore, content ourselves by briefly summarizing the more important data available as to the assimilation of sulphur and of phosphorus. These elements claim at least a word, since the former occurs in *all* proteids and the latter in certain of them.

The source of the *sulphur* in proteid is exclusively the sulphates absorbed by the root. The sulphates must certainly be reduced in the process of proteid synthesis, but where, and under what conditions this reduction takes place, we are quite ignorant. The same difficulty which we met with in discussing the assimilation of nitrogen meets us also in an even more pronounced form when we undertake an investigation into the mode of assimilation of sulphur ; for half of any proteid, roughly speaking, consists of carbon, 15–19 per cent. consists of nitrogen, but only 0·4 to about 2 per cent. consists of sulphur. If we write the formula of serumalbumin, as HOFMEISTER does, as $C_{450}H_{720}N_{116}S_6O_{140}$ (compare COHNHEIM, 1900), and assume that similar proteids also occur in plants, it is obvious that 75 atoms of carbon must be assimilated for every atom of sulphur. The consumption of sulphates in proteid synthesis must thus obviously be very limited. SCHIMPER (1890) considered that the assimilation of sulphuric acid also took place in presence of chlorophyll and under the influence of sunlight, but his assumption is by no means well founded, postulating as it does in general the same conditions as were pertinent to the assimilation of nitric acid.

Phosphorus also occurs in the molecules of certain proteid bodies ; it is absorbed only in the form of phosphate, and it would appear that the molecule of phosphoric acid becomes incorporated in the proteid molecule without essential modification, at least without any reduction. According to POSTERNAK (1900) the assimilation of phosphorus takes place in the leaf by the direct union of phosphoric acid and formaldehyde. The compound so produced, oxymethylphosphoric acid (H_3PO_4—CH_2O), POSTERNAK claims he has found in the plant, but it is questionable whether it is a first product of assimilation (compare IWANOFF, 1901 b). In addition to the proteids which contain phosphorus, nucleo-albumins and nucleo-proteids, phosphoric acid occurs in lecithins which contain no sulphur ; these latter bodies are very prevalent in plants (SCHULZE, 1894), and, according to STOKLASA (1893), may also arise in the chlorophylliferous leaf. Sulphur is, moreover, not limited to proteid, it occurs also in other substances of limited distribution, such as oil of mustard (C_3H_5NCS) in Cruciferae, allyl sulphide (C_3H_5S), in species of *Allium* ; nitrogen also is not confined to proteids and their anastates, but appears to be used in the construction of the widely distributed alkaloids and certain glucosides as well. As we know little or nothing as to the mode of formation of these bodies it is useless for us to study them in further detail at present.

Summarizing what we have learned from the last few lectures we may say : that the carbon-dioxide of the air is the only source of carbon available to green plants ; that they convert it into carbohydrates under the influence of sunlight and in the presence of chlorophyll, from which carbohydrates starch is produced as a product specially worthy of note ; that nitric acid is the chief source of nitrogen, and that that element in co-operation with the carbohydrates goes to form proteid especially. So far as we know, most cells can carry out synthesis of proteid without requiring sunlight as an essential condition of the process. It would also appear probable that a very large part of the proteid is formed in the leaf. In this capacity for assimilating carbon-dioxide and nitric acid the green plant stands in striking contrast to the higher animal, which is unable to construct either carbohydrate or proteid out of such simple compounds. It would be quite a mistake, however, to emphasize this

as a fundamental difference between plants and animals, since there are plants which are quite unable to construct proteids out of nitrates and others which require to be fed on carbohydrates previously synthesized. On the other hand it would appear probable that more accurate study of the lower animals will lead to the conclusion that the animal world also includes forms which more or less closely resemble green plants in so far as their nitrogen requirements are concerned.

Bibliography to Lecture XI.

BAUMANN. 1887. Versuchsstationen, 33, 247.
BERTHELOT and ANDRÉ. 1884. Compt. rend. 99, 683.
BOUSSINGAULT. 1860. Agronomie, Vol. I.
BOUSSINGAULT. 1861. Agronomie, Vol. II.
COHNHEIM. 1900. Chemie d. Eiweisskörper. Braunschweig. (ROSCOE-SCHORLEMMER, Lehrb. d. Chemie, Vol. IX.) [2nd ed. 1904.]
EMMERLING. 1884. Versuchsstat. 30, 109.
EMMERLING. 1887. Ibid. 34, 1.
EMMERLING. 1900. Ibid. 54, 215.
FRANK. 1888. Landw. Jahrb. 17, 421.
FRANK and OTTO. 1890. Ber. d. bot. Gesell. 8, 331.
[GODLEWSKI. 1903. Bull. Acad. de Cracovie, Math.-nat. Cl. p. 313.]
GREEN-WINDISCH. 1901. Die Enzyme. Berlin.
HAMMARSTEN. 1895. Lehrb. d. physiol. Chemie. Wiesbaden, 4th ed.
HANSTEEN. 1899. Jahrb. f. wiss. Bot. 33, 417.
HOFMEISTER. 1902. Ascher-Spiro, Ergebnisse d. Physiologie 1, 1 (Biochemie), 759.
IWANOFF. 1901, a. Versuchsstationen, 55, 78.
IWANOFF. 1901, b. Jahrb. f. wiss. Bot. 36, 355.
KERNER. 1887. Pflanzenleben, 1, 60.
KOSSEL. 1901. Ber. d. chem. Gesell. 34, 3214.
LAURENT, MARCHAL and CARPIAUX. 1896. Bull. Acad. Belg. III, 32, 12.
[LAURENT and MARCHAL. 1904. Bull. Acad. Bruxelles, Cl. d. Sc. p. 55.]
LIEBIG. 1840. D. organ. Chemie in ihrer Anwendung auf Agrikultur.
LUTZ. 1899. Annales Sc. nat. VII, 7, 1.
MALINIAK. 1900. Revue de Bot. 12, 337.
[MARCANO and MUNTZ, 1889. Compt. rend. Acad. Sc. Paris, 108, 1062.]
MAYER, AD. 1901. Agrikulturchemie, 5. Ed. Heidelberg.
MAZÉ. 1900. Annales Instit. Pasteur, 14, 26.
MOLISCH. 1887. Sitzungsber. Wiener Akad., Math.-nat. Cl. 95, 1, 221.
[MUNTZ. 1889. Compt. rend. Acad. Sc. Paris, 108, 900.]
PITSCH. 1887–96. Landw. Jahrb. 34, 217 ; 42, 1 ; 46, 357.
POSTERNAK. 1900. Revue gén. de Bot. 12, 5.
[REINHARD and SUSCHKOFF. 1905. Beihefte bot. Centrbl. 18, i, 133.]
REINKE. 1883. Quoted from Theoret. Biologie (1901), p. 236.
SCHIMPER. 1888. Bot. Ztg. 46, 65.
SCHIMPER. 1890. Flora, 73, 207.
SCHLÖSSING. 1874. Compt. rend. 78, 700.
SCHULZE. 1894. Versuchsstat. 43, 367.
SCHULZE. 1902. Ibid. 56, 97.
STOKLASA. 1898. Zeitschr. f. physiol. Chemie, 25, 398.
SUZUKI. 1898. Bot. Centrbl. 75, 289.
TREUB. 1895. Annales Jardin Buitenzorg, 13, 1.
[TREUB. 1905. Ibid. 2nd ser. 4, 86.]
[TREBOUX. 1904. Ber. d. bot. Gesell. 22, 570.]
WINTERSTEIN. 1901. Ber. d. bot. Gesell. 19, 326.
ZALESKI. 1900. Bot. Centrbl. 87, 277.
ZALESKI. 1901. Ber. d. bot. Gesell. 19, 331.
[ZALESKI. 1905. Ibid. 23, 126.]

LECTURE XII

THE CONVERSION OF THE PRODUCTS OF ASSIMILATION. I

THE DISSOLUTION OF STARCH IN SEEDS BY MEANS OF ENZYMES

CHLOROPHYLL is not developed in all the cells of the higher plant; in the petiole and most stems it is small in amount as compared with the colourless protoplasm, while subterranean parts, such as roots, rhizomes, &c., are quite free from it. Cells without chlorophyll, however, as we have seen, are entirely incapable of forming carbohydrates from carbon-dioxide, and consequently they are forced to obtain it from green cells. It necessarily follows that the products of assimilation in the leaf blade must migrate from it to undergo conversion elsewhere. This is probably true only of the carbohydrates, since, to all appearance, with their aid, cells free from chlorophyll are also capable of forming proteids in the dark. Should proteids also arise in the leaf to any great extent they too probably migrate. Migration of the products of assimilation out of the leaf may also be deduced from other evidence. Leaves in their earliest stages of development are colourless, and consequently must depend for their further growth on external supplies of organic material; at a later date, after the formation of chlorophyll, they begin to exhibit the phenomenon of carbon assimilation, and we must conclude that they employ the products of that assimilation in the first instance for their own construction. After a short time, however, a stage in development is reached when the leaf has attained its definite size, and then arises the question, what becomes of the products if they be not translocated? Experience teaches us that accumulation of starch in the confined limits of the chloroplast limits its assimilatory activity, but, as a matter of fact, such an injurious accumulation of starch does not take place under ordinary circumstances, because any excess undergoes translocation. We shall find in the next lecture that a leaf filled with starch may often lose it all in the course of a single night, and the fact that in *isolated* leaves no such disappearance of starch takes place demonstrates that this disappearance in the normal leaf is due not to a consumption of the starch *in situ*, but to its *transference* from the leaf to the stem by way of the petiole.

Starch is, however, solid, insoluble and incapable of migrating either actively or passively from cell to cell. Its translocation is possible only in the form of a soluble carbohydrate, after undergoing chemical alteration. There is no fact more clearly established in vegetable physiology than this, that a supply of soluble organic plasta passes from the assimilating leaf to regions of the plant which have themselves no power of bringing about carbon assimilation. A study of the transformations which these organic substances undergo, reveals to us certain functions performed by them, of which the following are the most important :—

1. The products of assimilation in the leaf blade act as *constructive materials*; they are transferred to wherever the plant is using these bodies—to the growing points of the stem and root and also to the cambium. In these situations, the organic materials manufactured by the leaf are devoted to the formation of new cells.

2. The products of assimilation act as *reserve substances*, either where they are formed or, after translocation, are stored up more or less permanently in other situations. Such reserves are afterwards converted into plasta and employed as constructive materials or for other purposes.

3. The products of assimilation are *oxidized*, and in consequence become once more altered into simple inorganic bodies such as are used by the leaf in the manufacture of organic compounds. Katabolic processes, such as these, are inseparable from all vital activities. Those substances which are sacrificed in this way may be termed *working materials*.

4. Since the conversion of materials is accompanied by translocation we

may consider, finally, *translocation products*. It must be noted, however, that this subdivision, based on the function of these substances in the plant, gives no indication whatever of the chemical nature of the compounds. The four different types of material *may* be chemically distinct but they need not; glucose, for example, may occur as a primary assimilation product, as a translocation compound, as a plastic substance, a reserve, or as a source of energy.

It would be obviously most natural to commence our study of the migration and translocation of the products of assimilation by observation of the method by which such substances are removed from the leaf; but for many reasons it is advisable to begin with the *reserves*, which are usually regarded as assimilatory products redeposited in 'secondary storehouses'. Reserves are deposited in these storehouses in such quantities that plants are often able at their expense, to develop to a considerable extent in darkness without needing to have recourse to any direct products of assimilation. Leaves, on the contrary, contain very little in the way of reserves, are rapidly deprived of them, and are liable to injury if kept in darkness for any length of time. Consequently the most important researches have been carried out on storehouses of reserves, and more especially on *seeds*. When we have mastered the conditions which prevail in these structures the transformations which take place in the foliage leaves will be easily understood.

The most important constituent of every seed is the embryo. It consists of a small, often microscopic young plant, in which we may distinguish one or two more or less well developed cotyledons, all other parts being still in an embryonic condition. Between the cotyledons may be distinguished the plumule, or growing point of the stem, surrounded by a few leaves, and, at the other end, the growing point of the root, the radicle. The whole embryo is in general enclosed in a special tissue, the endosperm, and that in turn by a seed-coat. When the seed is separated from the parent it cannot at once undergo development, for it is deficient in moisture, without which growth is impossible. In addition to certain other external factors which are conditions of germination, viz. warmth and oxygen, water is primarily essential; when that is supplied the seedling begins to grow. Generally speaking, the root bursts the seed-coat and imbeds itself in the soil; later on, the plumule is extended and gives rise to leaves above ground. As soon as the leaves develop a green colour on exposure to light, the plant becomes independent and can nourish itself by products which it itself has manufactured, but its entire development up to this stage is possible only if it be provided with reserves supplied to it by the parent. These reserves are as a rule capable of supplying all that is necessary for much later stages in the development of the seedling, so that from large seeds, such as those of the bean, plants of considerable dimensions may be produced in the dark, entirely at the cost of these reserves. The reserves are often deposited in the seedling itself, and the relatively bulky seed-leaves are frequently the seat of such deposition. The endosperm, however, a tissue external to the seedling may be the seat of deposit of such reserves. It is unnecessary for us to enter on the discussion of such purely morphological matters as the difference between endosperm and perisperm, nor need we concern ourselves with the question as to why some plants deposit their reserves wholly or in greater part in endosperm, or only in cotyledons or in both situations. For the purposes of physiology it is sufficient for us to know that such reserves are placed in the neighbourhood of the growing parts of the seedling. It is important, however, that we should become acquainted with the chemical nature of these reserves.

In seeds we meet with organic substances as well as minerals as reserves, and the former we recognize as of two kinds, one nitrogenous and the other non-nitrogenous. These three kinds of materials are not, however, always stored up in seeds in the proportions in which the young plant makes use of them. If that were the case the assimilated reserves at any given time

would be used up in equal proportions, as when germination takes place in darkness and when the seedling is provided with distilled water only. In nature, the root, as we have said, generally forces its way at once into the soil and draws therefrom the necessary minerals, and since its duty is to supply the seedling with such materials from the very first, naturally we need not expect more than a trace of minerals in the seed itself. This is the reason why GODLEWSKI (1879) found that seedlings of *Raphanus* developed far better in the dark when he provided them with nutrient salts than when he gave them distilled water only. It was only then that the seedlings could make full use of the organic reserves and so attain twice the weight they reached when grown in pure water. If excess of non-nitrogenous reserves be supplied and no nitrates be given, or if these bodies cannot be assimilated sufficiently in the dark, growth in the long run comes to a standstill. The converse is true of many Leguminosae ; although development is inhibited in darkness, one still finds in the seedlings nitrogenous organic substance accumulated in quantity out of all proportion to the amount of non-nitrogenous substances present. The degree of development is determined here also by the nutrient present in minimum quantity (compare Lecture VII, p. 83).

The reserves in seeds are bodies either entirely insoluble in water or characterized by having very large molecules (colloids). This has two advantages ; in the first place, substances containing no water take less room, and in the second, the high osmotic activity of concentrated solutions of crystalloids is avoided. We shall find that non-desiccated storehouses behave quite differently. The non-nitrogenous reserves which occur most commonly in seeds are *starch*, *cellulose*, and *fat*, the nitrogenous bodies are represented by *proteids*. In order to understand clearly how the dissolution and translocation of reserves is effected it will be most convenient for us to begin with a consideration of starch, for it is not only a very common reserve, but has also been the most thoroughly studied.

As already remarked, starch is insoluble in water unless chemically altered. Such alterations as give rise to soluble products may be effected, *apart from the plant*, in very various ways. Water at a high temperature acts in this way, causing starch to turn first of all into a paste and finally altering it into dextrin and dextrose. A similar decomposition is induced by mineral acids (e.g. hydrochloric acid), especially if these be warm. Other products are formed, however, when starch is dissolved in alkalis, calcium nitrate, chloralhydrate, &c. In the *plant*, starch, whether it be growing or whether it be dissolved, is surrounded by the chromatophore and cannot come in contact either with acids or alkalis ; its dissolution is effected in the plant by means of a substance with quite peculiar properties, viz. diastase, belonging to the physiological group of enzymes or ferments (compare SCHLEICHERT, 1893). Diastase is a product of the activity of the organism, but is capable of carrying out its functions apart from it. The most convenient method of obtaining diastase for study is to take some seeds containing abundant starch, such as barley, a short time after the commencement of germination, grind them down and extract them with water at a temperature of about 50° C. The diastase and other soluble bodies dissolve in the water, and we thus obtain a barley or malt extract for purposes of investigation.

On treating starch grains with this extract, we find that they gradually dissolve in precisely the same way as they do in the uninjured germinating seed (Fig. 32, p. 155). We may obtain a knowledge of the resulting products more readily by investigating the alteration effected in starch *paste*. Employing the iodine test we find that the original blue reaction rapidly gives place to a wine-red coloration. Finally, this latter reaction also disappears. Even without using iodine the fluid exhibits a marked alteration in *appearance*. Originally it is semi-fluid and opalescent ; now it becomes transparent and quite watery. The starch, as such, has disappeared, and dextrin and maltose

take its place (LINTNER and DÜLL, 1893; A. MEYER, 1895). Maltose betrays its presence by the fluid being capable of reducing alkaline copper sulphate (Fehling's solution).

We do not yet know exactly how the change into sugar is effected in all cases, but we must assume that the decomposition of starch is a gradual one, dextrin being formed first of all, which later is changed into maltose. By treatment with iodine it is often possible to distinguish a series of dextrins, but we are quite unable to say in what relation these stand to starch. They appear to have the same chemical composition as starch, differing from it only in the smaller size of their molecules. The dextrin molecule is still a large one in comparison to that of maltose, its molecular weight being about eighteen times as great. The formation of maltose appears to be effected according to the following equation :—

$$\underset{\text{Dextrin}}{C_{216}H_{360}O_{180}} + 18H_2O = 18\underset{\text{Maltose.}}{(C_{12}H_{22}O_{11})}.$$

On the absorption of water a *hydrolytic decomposition* takes place ; but it does not appear impossible that a similar hydrolysis occurs previously in the formation of dextrin itself.

The transformation of starch into sugar by the agency of malt extract can be demonstrated in a few minutes in a test-tube kept at suitable temperature. As in the case of germinating barley, so other germinating seeds yield diastase after digestion with water or glycerine ; moreover, diastase may also be shown to occur in many other amyliferous plant tissues as well as in digestive secretions in the animal body (saliva ; pancreatic secretion). There is no doubt, however, that diastase is by no means the same in character in each case ; indeed, profound differences have been discovered to exist, not merely as regards the products of the reaction but also with relation to the influence of external factors. To all appearance there are *several* kinds of diastase. It is very likely, for example, that the transformation of starch into dextrin is effected by a diastase differing from that which decomposes dextrin into maltose. By heating the malt extract to a temperature of about 80° C. (compare DUCLAUX, 1899, 400), its capacity for forming maltose is destroyed, although the formation of dextrin still continues. Further, the decomposition of dextrin does not always occur in the same way ; often maltose *only* is produced, at other times glucose appears as well. In the latter case a hydrolytic decomposition of the maltose molecule into two molecules of grape sugar takes place. Those diastases which produce *maltose only* may be distinguished from each other by their intermediate products (compare BEIJERINCK, 1895).

If we now compare the effect of diastases with that of a hydrochloric acid solution, it would appear that the former have a more limited activity than the latter. While *one acid* is sufficient to transform starch into glucose, *three different diastases* are required, each having a restricted but definite part to play in the total result. The same is true of other kinds of enzymes. The enzymes are thus much more delicate agents than the acids, and to this is due the importance attached to them in modern physiological chemistry.

Inquiring now into the chemical characters of diastase, we have first of all to note that our malt extract is by no means a pure solution of diastase, for as yet it has not been found possible to isolate it completely from the other constituents of the extract. If we add alcohol to the solution a precipitate is obtained which gives the proteid reaction, and which, when dissolved in water, exhibits the same power of dissolving starch that diastase has, though to a rather less degree. If this solution be heated above 80° C., proteid, and with it the diastase, separate out ; the latter no longer, however, has the power of dissolving starch. It might be thought, therefore, that diastase was a proteid which is coagulable at high temperatures, but this view cannot be taken as *proved*, for diastase might have, chemically, nothing to do with the proteid. It might

be a body of quite unknown composition, not as yet isolated from the proteid. Since very small quantities of diastase possess great hydrolytic powers, the actual diastase itself may form merely a trifling impurity in the proteid obtained by alcoholic precipitation from the malt extract.

What has especially attracted the attention of physiologists to diastase is, on the one hand, its behaviour at different temperatures, and on the other, its action on certain substances. At 0° C. the dissolving action of diastase on starch is scarcely noticeable ; an increase in temperature is followed by a rapid increase in its activity, until at 50° C. it reaches a maximum maintained till 63° C. is reached ; if it be heated still further, the activity of diastase again decreases, until finally, at about 85° C., its power becomes destroyed (KJELDAHL, 1879). If we construct a graphic curve (Fig. 30) whose abscissae indicate degrees in temperature and whose ordinates show the amount of starch dissolution effected by the diastase, it will be found that the curve bears a strong likeness to those other curves which express the dependence of various functions of the *living plasma* on temperature, such as we have still to study in growth and movement, and which we have already seen in the case of carbon assimilation. The maximum, minimum, and optimum points of this curve given by different diastases are not always coincident (LINTNER and ECKHARDT, 1890). The diastatic curves differ from other physiological curves chiefly in the fact that the optimum point always stands very high ; indeed, it stands so high that it is never reached in the plant, since at 50°–60° C. carbon assimilation is impossible and generally the limits of life itself are reached or exceeded before the optimum effect is obtained.

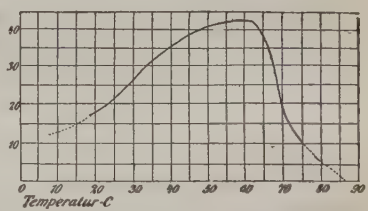


Fig. 30. Dependence of the activity of malt diastase on temperature. After KJELDAHL (1879).

In considering the influence on diastase of certain substances we will begin with those which tend to *retard* its activity, and which act on diastase just as poisons do on protoplasm. According to BOKORNY (1901), formaldehyde is to be considered in this light, since, even in a concentration of 0·01 per cent., it affects both protoplasm and diastase injuriously, after it has operated for a certain time. Diastase, however, responds to the majority of poisons in a different manner from protoplasm, viz. in being much less sensitive to them. While the latter is destroyed by very minute quantities of corrosive sublimate (0·00005 per cent.) and silver nitrate (0.000001 per cent.), a concentration of 0·01 per cent. in both cases is required to produce a poisonous effect on diastase. We know also that by introducing certain poisons in sufficient quantity it is possible to kill micro-organisms, and yet the enzyme remains fully active. Since, however, micro-organisms, as we shall see by and by, can greatly influence experiments with malt extract, their exclusion becomes of very great significance. Thymol or chloroform is usually employed for this purpose, not corrosive sublimate.

In contrast to these *inhibitory* poisons, other substances are known which act in the highest degree as *accelerating* agents. Generally speaking, all additions of free mineral acids act in this way, if they be present in traces only ; so also do neutral salts (e. g. sodium chloride) in somewhat larger doses, and finally, salts of aluminium, phosphoric acid compounds, and asparagin in sufficiently high concentrations. Thus EFFRONT (cited by GREEN, 1901) found that in a certain time, the following unequal amounts of maltose were produced by a malt extract from starch paste :—

1. Without any addition	. . . . .	8·63	maltose
2. With addition of	0·5 %	calcium phosphate	46·12	,,
3. ,, ,,	0·25 %	ammonium alum	56·30	,,
4. ,, ,,	0·25 %	aluminium acetate	62·40	,,
5. ,, ,,	0·05 %	asparagin . .	61·20	,,

HANSTEEN (p. 144) has drawn certain conclusions as to the formation of proteid from the disappearance of starch on the addition of asparagin; these researches show, however, that he was only dealing with an acceleration of the diastatic activity by asparagin.

Whether such an accelerating agent be present or not, a solution of diastase is unable to transform the whole of the starch into maltose—usually a certain amount remains in the form of dextrin. Doubtless the reason for this is not that the diastase is used up after the dissolution of a certain quantity of starch, but that its power of transforming starch into sugar is inhibited by the accumulation of the products of the reaction. If adequate arrangements be made for the removal of the sugar formed, all the dextrin is finally turned into maltose, and theoretically only a small amount of diastase is necessary to transform an unlimited quantity of starch, without its losing its diastatic power in the process.

Quite a number of peculiarities, which we have now recognized as belonging to the diastases, are found in other substances formed by the organism, and to these bodies has been given the name of enzymes or ferments. Those with which we are at present concerned induce *hydrolytic decompositions*; later on we shall have to study enzymes which bring about decompositions otherwise than by hydrolysis (Lecture XVI). These enzymes act in very small quantities and take no part, or at least no permanent part, in the reaction. The reaction is always incomplete and may be accelerated or retarded by certain substances; their activity is dependent on temperature in the same way as we have seen that of diastase to be. The enzymes may be extracted from the organism by water or by glycerine, from which media they may be precipitated by alcohol.

Each individual enzyme apparently attacks only one or at most a few related bodies. We may distinguish at least five classes of enzymes, although apparently their number is much greater and their spheres of operation much more limited:—

1. *Amylases*, or diastases, which transform starch into sugar.
2. *Cytases*, which manufacture sugar from cellulose and the other carbohydrates associated with it in forming the cell-wall.
3. *Invertases*, which change disaccharides into monosaccharides, e. g. cane sugar into dextrose and levulose, maltose into two molecules of dextrose.
4. *Lipases*, which break up fats into glycerine and fatty acids.
5. *Proteases*, which act on proteids and produce from them diffusible bodies already enumerated elsewhere (p. 140).

In addition to these *specific* (hydrolytic) activities, the enzymes (all?) possess the power of splitting off oxygen from peroxide of hydrogen. In many respects, more especially in their dependence on temperature and many chemicals, the enzymes resemble organisms themselves, and it has for long been the custom to regard them as portions of the protoplasm, or, at least, as very highly complex substances. But it is by no means necessary to make such an assumption, since enzymes resemble in many ways a series of inorganic bodies which possess peculiar characteristics, and which are known as *catalytic agents*, and it is now the custom more than ever to regard the activities of enzymes as *catalytic* in their nature.

Catalytic agents are those which alter the rate of a reaction without themselves entering into the final product (OSTWALD, 1902). The catalytes which specially interest us for the moment are those which *accelerate* reactions. As a type of such catalysis we may take the decomposition of hydrogen-peroxide into oxygen and water in the presence of finely divided metals. Peroxide of hydrogen, it is true, also decomposes spontaneously, but the separation of oxygen takes place much more rapidly in presence of the metal, a very small quantity of the catalyte decomposing a very large amount of the peroxide without suffering any loss of power in the process. But the catalytic value of the metal is entirely dependent on its finely divided condition; platinum wire

is quite useless for this purpose, but platinum black or so-called colloidal platinum is, on the other hand, exceedingly active. BREDIG (1901) has obtained such colloidal solutions by electric spraying. Platinum and other metals may be broken up by kathodic spraying under water into particles so fine that they remain in suspension in the water, and cannot be seen even with the best microscope; such a fluid we term a colloidal solution. BREDIG has made a number of experiments with such solutions, which are of extreme interest to the physiologist. He was able, in the first place, to estimate more exactly than previously the amount of platinum necessary, and showed what was the minimum amount which could act catalytically on hydrogen-peroxide, viz. 0·000003 mg. From his further researches the catalytic effect of certain substances may be estimated which are known to be inhibitory. Minute quantities of sulphuretted hydrogen, bisulphide of carbon, hydrocyanic acid, &c., destroy the catalytic power of the platinum solution, but they do not do so permanently; after removal of the substance the catalysis begins anew.

BREDIG finds a strong likeness to exist between enzymes and such colloidal metal solutions, so much so, indeed, that he terms the latter 'inorganic enzymes'. This likeness is expressed in their colloidal form, their mode of action, and the effect produced upon them by the substances named. We must leave as a debatable question whether we may correctly designate the suspension of platinum particles in water as a colloid corresponding to organic substances capable of swelling and confine ourselves to the consideration of the other two points of comparison. In addition to the catalytic action of apparently all of them on hydrogen-peroxide 'enzymes have over and above specific effects on definite substances, but these specific effects do not appear to us to be as yet proved to be possessed by a colloidal solution of platinum (compare Zeitschr. f. phys. Chem. 31, 262, note). The specific hydrolytic action of the enzyme cannot have anything to do with the decomposition of hydrogen-peroxide; the one action may be differentiated from the other by heating to a certain temperature (JACOBSON, 1892). Pancreatic secretion for example after heating to a temperature of 61° C. can still transform starch into sugar, but it can no longer abstract oxygen from peroxide of hydrogen. It is very obvious that by this means we have separated out a substance which, in its behaviour it is true, shows a strong resemblance to colloidal platinum, and that the *actual* enzyme remains uninjured. We arrive at similar conclusions on a closer analysis of the action of the poisons mentioned above; of these, hydrocyanic acid is especially a case in point, because it acts poisonously on enzymes in the same way as it does on colloidal platinum. In reality, the hydrocyanic acid affects *only* the activity of the *impure* enzyme on peroxide of hydrogen, and leaves the *specific action* of the enzyme quite *intact* (JACOBSON, 1892). Further differences between BREDIG'S 'inorganic enzymes' and organic enzymes may perhaps come out on a closer study of the effect of temperature on the reactions. At present, at all events, it is by no means certain whether the so-called inorganic enzymes show a temperature curve with minimum, optimum, and maximum points, but if this be considered of secondary importance we have, at least, one other difference of greater weight, i. e. the close of the reaction. The platinum solution remains active as long as a trace of peroxide is present; in other words, the reaction is complete; in the case of enzymes, however, the reaction (see p. 152) is incomplete unless the products be withdrawn. It would not be out of place here to go into the question of the incompleteness of the enzyme reaction, but it is possible in the present condition of the science only to draw attention to the diametrically opposite views advanced on the question, and on which no decision has as yet been reached.

The reason for the incompleteness of the reaction generally lies in this, that it does not consist of *one* reaction only, but of *two* processes, which induce opposite changes and which lead to an equilibrium at a definite temperature.

Thus, in the hydrolytic decomposition of an ester by hydrochloric acid, alcohol and acid are formed, but the alcohol unites again with the acid, water being given off, and an equilibrium is brought about if the formation of the ester goes on as rapidly as its decomposition. If enzymes behave in the same way as the hydrochloric acid in this example, then they must be able to induce not only a hydrolysis, but, under certain conditions, a synthesis also. Something like this has been observed by HILL (1898). He obtained an enzyme from yeast, which changes maltose into dextrose, allowed it to act on a 40 per cent. solution of dextrose, and found that after a long time 14·5 per cent. of the dextrose was changed into maltose. A synthesis took place when water was withdrawn, and an equilibrium was reached when 14·5 per cent. of the sugar was composed of maltose and 85·5 per cent. of dextrose. The condition of equilibrium depended essentially on the concentration of the solution, as the following summary shows :—

Amount of dextrose originally present.	Amount of dextrose after the action of the enzyme.	Amount of maltose after the action of the enzyme.
40 %	85·5 %	14·5 %
20 ,,	90·5 ,,	9·5 ,,
10 ,,	94·5 ,,	5·5 ,,
4 ,,	98·0 ,,	2·0 ,,
2 ,,	99·0 ,,	1·0 ,,

The more dilute the solution of dextrose the *less* the amount of maltose formed. HILL's work has been confirmed in many respects by WENT (1901), and a *reversible action* has been established in the case of other enzymes as well (lipase, HANRIOT; maltase, EMMERLING, 1901). In spite of this reversible action, it is quite obvious that we, as a rule, see only one aspect of the enzymic activity, i. e. hydrolysis, and that when the products of the reaction have been effectively removed, a *complete* hydrolysis of the products of the process will be found to have taken place.

The view advanced by HILL is opposed to that taken by TAMMANN (1892). According to this investigator *no* reversible action occurs during enzymic activity. He holds that when hydrolysis remains *incomplete*, the reason is that the enzyme becomes altered into an inactive variety under the influence of the accumulation of decomposition products. Further research is needed to determine which view, HILL's or TAMMANN's, is the right one ; but if, as we have no doubt, HILL's theory be correct, then BREDIG would have to show that a *formation* of hydrogen-peroxide took place in presence of his 'inorganic ferments', if he desired to establish a comparison between these bodies and organic ferments.

The similarity between these substances does not seem to us to be very great, and to be confined to the fact that enzymes and platinum solutions are catalytic agents. Catalytic substances, however, belong to very diverse categories (OSTWALD, 1902). We might, indeed, doubt whether enzymes are catalytic agents at all, seeing that many of the decompositions in question are not perceptible in the absence of the enzyme. Starch, for example, under ordinary conditions, produces no maltose in water; and it would appear doubtful whether we are entitled to regard the action of the enzyme merely as a case of acceleration of a previously existent process. If we find, however, that hydrolysis certainly occurs at *higher* temperatures *without* an enzyme being present, and that the reaction gradually ceases when the temperature is lowered, we cannot deny spontaneous hydrolysis at ordinary temperatures, even if the products arising from the action do not make themselves apparent perhaps till years afterwards. At all events the classification of enzymes in the category of catalytic agents is the best hypothesis we can put forward at present.

After these general remarks on enzymes and on diastase in particular, let us return to the consideration of the germination of seeds. Dissolution of starch may be studied best in the seeds of Gramineae, which possess an especially

abundant supply of this reserve (about 80 per cent. of the dry weight). Fig. 31 shows a longitudinal section through the lower end of a grain of wheat. Within the testa, which is in this case fused with the wall of the fruit, we observe the embryo (*Em*), well developed and possessing a special organ, the so-called scutellum (*Sc*), lying against the abundant endosperm (*End*). The contents of the cells of the endosperm are not homogeneous. A single layer of peripheral cells (the aleurone layer, *Al*) containing aleurone grains, lies immediately within the testa, while the large central mass is packed with starch. Diastase may be demonstrated even in the resting endosperm, and it becomes very noticeable when water is absorbed at the beginning of germination. It produces a dissolution of the starch which may become very extensive if the maltose which arises in the process be removed. In normal germination this is effected by the seedling absorbing greedily the sugar presented to it by means of the superficial layer of the scutellum. If the embryo be removed, not only does the translocation of the sugar come to an end, but its formation also ceases and the starch grains remain intact. HANSTEEN (1894) and PURIEWITSCH (1897) were able to show that an emptying of the endosperm cells took place in the absence of an embryo, if the seed were placed in contact with a large quantity of water

Fig. 31. Longitudinal section through the lower part of a grain of wheat; *End*, endosperm, *Al*, aleurone layer, *Em*, embryo, *Sc*, scutellum. After SACHS (1862), slightly magnified.

Fig. 32. Starch grains from germinating barley. 1–4, successive stages in the dissolution of the grain. From the Bonn Textbook.

with due antiseptic precautions, in such a way that only a small part was submerged. The experiment was so arranged that in place of the embryo a plug of gypsum was applied to the endosperm where the scutellum had been, its lower part being in contact with water. It was found in this way that various grass seeds exhibited after about a week many corroded starch grains in the endosperm (Fig. 32); after eight to fourteen days most of the cells were completely emptied, and a sugar, which was capable of reducing Fehling's solution, could be demonstrated in the water which removed it from the endosperm. Whether this sugar corresponded in amount to the starch which had disappeared seems not to have been determined, but obviously the determination of this point is of great importance in coming to a decision on the question. Further, a complete evacuation of the starch in PURIEWITSCH'S experiments took a much longer time than in the case of the normal seedling. It is not probable that the imperfect removal of the sugar formed was the cause of this, although no other explanation is available at present. It has been clearly proved (LINZ, 1896) that the scutellum contains at any time more (or more active) diastase than the endosperm, so that it may be that in normal germination diastase from the scutellum penetrates the endosperm and there assists in the dissolution of the starch. Although it cannot be doubted that

such an excretion of diastase does take place from cells, and although it has been shown that it is a phenomenon specially characteristic of the grass embryo, e.g. by BROWN and MORRIS (1890), it cannot be said that the evidence for it is altogether above criticism (LINZ, 1896; GRÜSS, 1897).

As noted above, a reducing sugar was always found in the fluid used in the experiments where the endosperms of grasses were allowed to undergo transformation in absence of an embryo; in addition, however, cane sugar also appears in not inconsiderable quantity, that is to say, another disaccharide capable of reducing only after treatment with hot acids. This sugar, according to our present knowledge, cannot be produced by hydrolysis of starch, and so its appearance requires to be explained. At present we must be content to believe that it is present as such in the seed, a view not entirely improbable, seeing that SCHULZE (1899) has found cane sugar in seeds of the Gramineae. Again PURIEWITSCH has shown, from other investigations, that the emptying of the endosperm is not due simply to diffusion. He agrees with HANSTEEN in believing that the emptying takes place more rapidly if large quantities of water be supplied with the view of dissolving the sugar. The explanation seems simple enough: diffusion can take place only so long as the fluid outside is less concentrated than that in the cells; for if the sugar formed in the cells cannot diffuse out then the hydrolysis of starch ceases. Thus PURIEWITSCH found that far less starch was dissolved from the endosperm when he used in place of water a 1–3 per cent. solution of cane sugar or dextrose. Although it has not been definitely proved to be the case that dextrose is actually formed from starch, one may still regard these two sugars as the *direct* cause of the inhibition of diffusion. The situation is quite altered when glycerine, potassium nitrate, and sodium chloride are used, for PURIEWITSCH found that these substances vigorously inhibited the dissolution of starch. It is possible to explain this result *physically*, but one may doubt the purely physical action of cane sugar and dextrose, and we must remember, further, that the cells of the endosperm are living, and as such are affected in a variety of ways by the environment. Sufficient evidence is not forthcoming to show how it is that organisms are able to inhibit the action of an enzyme, but we do know of many facts which prove to us that they have this power. PURIEWITSCH, for example, found that the removal of starch ceased when he supplied the endosperm (in the absence of the seedling) with air free from oxygen, or with air containing chloroform; and yet we know that, apart from the cell, a solution of diastase acts equally well on starch whether oxygen or chloroform be present or not. Experiments such as these are full of lessons for us. It is only right that great stress should be laid on the study of enzymes in modern physiological chemistry, since these bodies obviously have important functions to perform in the organism; but we must not hope for too great results from such studies. We may learn in this way what reagents the living cell uses in dealing with these substances, and so imitate in many cases what goes on in the cell, by reproducing in test-tubes the chemical transformations that go on in the organism, with the aid of acids or enzymes; but these transformations are not those which are characteristic of the organism; the secret lies in *controlling* the enzyme so that at one moment it is active, at another it is quiescent. Further, we can give no general answer to the question how an accumulation of the products of the reaction interferes with the activity of the enzyme. Probably substances acting as inhibiting agents or poisons to the enzyme play a principal part in retarding enzymic activity (compare CZAPEK, 1903), but as yet we are unable to understand how the organism governs the production at the same time of an enzyme and an anti-enzyme. It cannot be doubted, however, that such a co-ordination or *appropriate* production of these bodies really takes place. Similar cases of regulation of secretions occur *everywhere* in organisms, as we shall find later on.

Bibliography to Lecture XII.

BEIJERINCK. 1895. Centrbl. Bakt., Abt. II, 1, 221.
BOKORNY. 1901. Bot. Centrbl. 85, 293.
BREDIG. 1901. Anorganische Fermente. Leipzig.
BREDIG. 1902. Ergebnisse der Physiologie (Spiro-Ascher), 1.
BROWN and MORRIS. 1890. Jour. Chem. Soc. Trans. 57, 458.
CZAPEK. 1903. Ber. d. bot. Gesell. 21, 229.
DUCLAUX. 1899. Traité de Microbiologie, II. Diastases. Paris.
EMMERLING. 1901. Ber. d. chem. Gesell. 34, 600 and 380.
GODLEWSKI. 1879. Bot. Ztg. 37, 97.
GREEN, J. R. 1901. Die Enzyme (German edition by Windisch). Berlin.
GRÜSS. 1897. Jahrb. f. wiss. Bot. 30, 645.
HANRIOT. 1901. Compt. rend. Paris, 132, 146.
HANSTEEN. 1894. Flora, 79, 419.
HILL. 1898. Journ. Chem. Soc. Trans. 73, 634.
JACOBSON. 1892. Zeit. für physiol. Chem. 16, 340.
KJELDAHL. 1879. Meddelelser fra Carlsberg Labor. 1, 121.
LINTNER and DÜLL. 1893. Ber. d. chem. Gesell. 26, 2533.
LINTNER and ECKHARDT. 1890. Quoted in Koch's Jahresbericht über Gährungsorganismen.
LINZ. 1896. Jahrb. f. wiss. Bot. 29, 267.
MEYER, A. 1895. Die Stärkekörner. Jena.
OSTWALD. 1902. Verhandl. d. Gesell. d. Naturforscher zu Hamburg 1901, Leipzig 1902.
PURIEWITSCH. 1897. Jahrb. f. wiss. Bot. 31, 1.
SCHLEICHERT. 1893. Nova Acta Acad. Leopold. 62, 1.
SCHULZE. 1899. Zeit. f. physiol. Chem. 27, 267.
TAMMANN. 1892. Zeit. f. physiol. Chem. 16, 271.
WENT. 1901. Jahrb. f. wiss. Bot. 36, 611.

LECTURE XIII

THE CONVERSION OF THE PRODUCTS OF ASSIMILATION. II

DISSOLUTION OF THE REMAINING RESERVES IN SEEDS
OTHER STOREHOUSES OF RESERVES

IN the course of their researches on starch dissolution in germinating barley, BROWN and MORRIS (1890) found that the cell-walls of the endosperm were also dissolved during germination. The cell-walls of the endosperm of barley are relatively thin, and hence the sugar resulting from their dissolution cannot play any very essential part in the nutrition of the seedling. Probably the significance of the absorption of the cell-walls in this case lies merely in the fact that the other enzymes are thus enabled to enter the cells more readily. The walls of the endosperm cells of other seeds, on the contrary, are remarkably thick, as, for example, in palms and many other Monocotyledons, and here also a dissolution of the cell-walls takes place in germination, so that it is quite legitimate to consider the materials of which they are composed as reserves, particularly as other carbohydrates are absent or present only in small quantity. The chemical composition of the cell-wall is still imperfectly known. At least two groups of substances take part in its formation—pectins and celluloses. The former of these we need not discuss at present since the most divergent views are held as to their chemical characters (SCHRÖDER, 1901 [compare CZAPEK, Biochemie, I, 545]). The celluloses on treatment with acids give rise by hydrolysis to various types of sugar, dextrose, mannose, galactose, and, following E. SCHULZE (1890–92), we may regard them as anhydrides of these hexoses as well as of certain pentoses (arabinose and xylose). The cell-wall only rarely consists of a single chemical compound ; in most cases it is formed of a mixture of several. Such celluloses are deposited as reserves in seeds especially, giving

rise to mannose and galactose in quantity but only to a little dextrose, on treatment with dilute acids, but only if the acids be *dilute*. SCHULZE terms them hemicelluloses in contrast to the true celluloses, which are capable of hydrolysis only by the action of concentrated acids. These latter can obviously be dissolved by the *plant* with great difficulty and never take any further part in metabolism.

The celluloses are perhaps better characterized by their behaviour in the presence of enzymes than in the presence of acids. Not only is this of more importance biologically, but it gives us also a clearer insight into their chemical nature. It is well known that the hemicelluloses are dissolved by enzymes in seeds, although we have no precise knowledge of these enzymes. BROWN and MORRIS (1890), from germinating barley, and NEWCOMBE (1889), from the cotyledons of lupins, as well as from the endosperm and the cotyledons of *Phoenix*, by the same method used in the extraction of diastase, have obtained a soluble enzyme which dissolves the cell-walls of the endosperm of barley rapidly, and more slowly the reserve cellulose of lupins. Although this power of dissolving the cell-wall has been attributed for several reasons to *diastase*, which as a matter of fact is never absent from such extracts, NEWCOMBE was able to prove conclusively the presence of a special enzyme, *cytase*. Although it has not been as yet possible to separate this enzyme from diastase, still NEWCOMBE's conclusion would appear fully justified because the amylolytic and cytolytic capabilities of the extract are not at all proportional to each other, the extract of lupins and of *Phoenix* having a very vigorous action on cellulose and a feeble action on starch, while malt extract acts conversely.

The distribution of the cytases has not as yet been fully determined, but it may be assumed that they occur wherever cellulose requires to be dissolved. That they frequently cannot be recognized may be accounted for by the fact that they are present only in *very small quantities*; indeed we find that the endosperm of palms, for example, takes a much longer time to dissolve than that of the Gramineae. [HÉRISSEY (1903) has shown that cytases are widely distributed, and that there are many types of them, each with its own specific activity.]

In addition to starch and cellulose a third non-nitrogenous substance occurs in seeds, viz. fatty oil. Fatty oils occur not in seeds alone but in all cells; indeed it is very doubtful whether there is such a thing as protoplasm free from fat. It is a normal component of the chloroplasts of certain plants, and was in such cases for long believed to replace starch as the final product in the process of carbon assimilation. More exact investigations (HOLLE, 1877, GODLEWSKI, 1877) do not confirm this view. It seems much more likely that the oil globules occurring in chloroplasts are not to be considered as products of assimilation, at least in the higher plants (although in *Vaucheria* FLEISSIG (1900) holds that fat does take the place of starch), and that they are not capable of further conversion. It is for that reason we have made no mention of fats in the preceding pages. In seeds, fats are undoubtedly reserves, and in many seeds they form the chief part of them, as may be seen from the following table :—

	Water.	Nitrogenous matter.	Fat.	Non-nitrogenous extractives.	Wood.	Ash.
Almond . . .	5·39	24·18	53·68	7·23	6·56	2·96
Hazel-nut . .	3·77	15·62	66·47	9·03	3·28	1·83
Poppy-seed . .	5·79	14·09	47·69	18·74	5·76	7·93
Coco-nut . . .	5·81	8·88	67·00	12·44	4·06	1·81

We must treat of the fats in this section, although we cannot enter into a discussion of their mode of origin at the present moment.

The fats are really glycerine esters of various fatty acids, belonging to one or other of three series. The first series has a composition represented by the

formula $C_nH_{2n}O_2$, the second, $C_nH_{2n-2}O_2$, and the third, $C_nH_{2n-4}O_2$. To the first group belong laurinic acid ($C_{12}H_{24}O_2$), myristic acid ($C_{14}H_{28}O_2$), palmitic acid ($C_{16}H_{32}O_2$), stearic acid ($C_{18}H_{36}O_2$), and arachidic acid ($C_{20}H_{40}O_2$); to the second series belong hypogaeic acid ($C_{16}H_{30}O_2$), oleic acid ($C_{18}H_{34}O_2$), and brassidic or erucic acid ($C_{22}H_{42}O_2$); to the last group, linoleic acid ($C_{16}H_{28}O_2$) (compare SCHMIDT, 1891). [An enumeration of all the fats which occur in seeds will be found in CZAPEK's Biochemie, I.] The glycerine esters of these fatty acids are briefly described as palmitin, stearin, olein, &c., and they are to be considered as formed from glycerine and a fatty acid by withdrawal of water, and conversely they may, by absorption of water, be decomposed into glycerine and a fatty acid :—

$$\underset{\text{Palmitin}}{C_3H_5(C_{16}H_{31}O_2)_3} + 3H_2O = \underset{\text{Glycerine}}{C_3H_5(OH)_3} + 3\underset{\text{Palmitic acid.}}{(C_{16}H_{32}O_2)}.$$

Such a decomposition, which we may obviously consider as a case of hydrolysis, actually takes place in the course of germination. According to R. H. SCHMIDT (1891), germinating seeds contain an appreciable quantity of free fatty acid, amounting to from 10 per cent. to 30 per cent., and there can be no doubt that its occurrence is due to the action of an enzyme, lipase. GREEN (1890) extracted such an enzyme from seeds of *Ricinus*, and was able by its means to obtain glycerine and a free fatty acid from castor oil apart altogether from the seed. The presence of glycerine, however, has not hitherto been demonstrated in the germinating seed, obviously because it rapidly undergoes transformation and migrates easily from cell to cell. Fatty acids, until a short time ago, appeared to be incapable of translocation from cell to cell; the cell-wall, saturated with water, was held to form an impassable barrier to any such movement. According to R. H. SCHMIDT, this is perfectly true of artificial cellulose walls, although not for the walls of living cells, which allow fats to penetrate them in considerable quantity, more especially if the walls contain a certain amount of free acid. It may be supposed that some substance present in the cell-wall unites with the free fatty acid to form a soap, and that this soap permeates the cell-wall and thus permits the fat to pass through. This takes place all the more readily if the oil be subdivided into very minute drops, that is to say, is emulsified, and the fatty acids are well known to have an emulsifying power.

In addition to *Ricinus*, several other seeds, such as those of rape, poppy, and hemp, have been shown by SIGMUND (1890–2) to contain a fat-splitting enzyme, so that we have every right to assume the general distribution of fatty oils and the corresponding frequency of lipase. More detailed investigations on this subject are not as yet forthcoming, and we know still less as to the process which microscopical research has established beyond a doubt (SACHS, 1859), but which, from the purely chemical point of view, is incomprehensible, viz. the transformation of fats into sugar. The amount of sugar present in germinating oily seeds renders it impossible that it has been derived from glycerine only; the fatty acids must obviously also contribute to the formation of carbohydrates (compare Lecture XIV).

In addition to non-nitrogenous we also meet with nitrogenous reserves deposited in seeds in the form of *proteid*; the relative proportion of nitrogenous and non-nitrogenous substances is in the highest degree variable. Whilst in general the non-nitrogenous substances are predominant, there are many plants, more especially the Leguminosae, which contain a very large percentage of nitrogenous reserves. A glance at the following table (KÖNIG, 1882) demonstrates very effectively this variation :—

Seed.	Per cent. of nitrogenous substance in dry weight.
Rice (undecorticated)	6·49
Wheat	14·30
Kidney beans	29·94
Linseed	29·32

Proteid, in so far as it is a reserve substance and not a constituent of the protoplasm or nucleus, occurs in the endosperm as well as in the cotyledons in a definite morphological form, as aleurone. The aleurone grains originate as vacuoles in the protoplasm of the storage cells, which are always richer in proteid and poorer in water, and finally become solid bodies by desiccation. Owing to loss of water the various substances present in the vacuole separate out; certain proteid bodies appear in the crystalline form, other more complicated substances form spherical secretions, and both are enclosed in a coagulated ground substance; the constituents of the aleurone body thus enclosed are known under the names of crystalloid and globoid. From the chemical point of view, the most important researches on these bodies are those of WEYL (1877), SCHMIEDEBERG (1877), GRÜBLER (1881), also those of CHITTENDEN, OSBORNE, and their pupils (summarized by GRIESSMAYER, 1897); more recently TSCHIRCH (1900) has checked the results arrived at by these American investigators by micro-chemical methods. From this it would appear that the aleurone grains are composed of *globulins*. The percentage composition of the crystalloids of the brazil nut is, according to WEYL :—

C, 52·43; H, 7·12; N, 18·1; S, 0·55; O, 21·3.

while GRÜBLER gives as the analysis of the crystalloid of the cucumber :—

C, 53·21; H, 7·22; N, 19·22; S, 1·07; O, 19·10.

OSBORNE has identified a large number of globulins in the *crystalloids* of different aleurone grains, and has given them special names, and has also shown that the individual crystalloid is composed of several globulins. The differences existing between these are of no special interest to the physiologist, so that we need not enter into a discussion of them here. The *globoids* appear also to be composed of globulins, but these are united with calcium, magnesium, and phosphoric acid, and hence are very varied in character; they must be reckoned among the '*nucleo-albumins*' or the '*proteids*'. Finally, the *ground substance* of the aleurone body consists of globulins, mixed in all probability with albumoses.

During germination these reserve proteids must be altered into forms capable of undergoing diosmosis and migrating from cell to cell. Simple solution would not achieve this end without chemical alteration taking place at the same time; the large molecules must be broken up, and this is effected, as we know, by proteolytic enzymes (proteases). These enzymes have been much more thoroughly investigated from the animal than from the vegetable side, still the results which have been obtained may, without hesitation, be considered as applicable to the vegetable kingdom. Two types of proteases are known, which differ both so far as regards the conditions under which they operate, and also in the products to which they give rise. To the first type belong the *pepsins*, which act only in an acid solution, decomposing the proteids only into albumoses and peptones and in this way rendering them diffusible. Pepsin in the animal is produced in the stomach, while *trypsin*, the other type of protease, is formed in the pancreas. Trypsin differs from pepsin in the first place in operating best in an alkaline solution (1 per cent. soda), and also in inducing a much more thorough decomposition in the substances which it attacks. The albumoses and peptones, which are the first products submitted to its action, are broken down still further into the amido-compounds we have already referred to, and more especially into arginin, histidin, lysin, and ammonia. [In addition to pepsin and trypsin, a ferment erepsin has also recently been identified as of very widespread occurrence. The final products are the same as those of trypsin, but it attacks peptone only, never proteid. Its occurrence in seeds has not as yet been proved. (VINES, 1905).] It would appear that trypsin, or an enzyme like it, is of very

general occurrence in nature; in the plant world pepsins seem to be entirely absent. Since, in the germination of seeds with abundant proteid, amino-acids, or other similar substances produced by the action of the pancreatic secretion, may be recognized, more especially if by cultivation in darkness an accumulation of these bodies is induced (Lecture XIV), we may legitimately conclude the presence of a trypsin-like enzyme in the germinating seed. A ferment of this kind has been discovered by GREEN (1887) in lupins and *Ricinus* (1890), and by NEUMEISTER (1894) in barley, poppy, wheat, maize and rape. NEUMEISTER, however, searched in vain in many other seeds for a protease, although one would have expected to find such enzymes most readily in seeds, such as those of many Leguminosae (lupin, vetch, pea), containing large quantities of proteid. BUTKEWITSCH (1900) has fully confirmed GREEN'S work on the lupin in opposition to NEUMEISTER'S conclusions, and was able to show conclusively that leucin and tyrosin were formed from proteids of Leguminosae by acting on the latter with a glycerine extract of germinating seeds. WINDISCH and SCHELLHORN (compare GREEN, 1901) have also discovered a trypsin-like protease in germinating barley. There is thus nothing to prevent us assuming the presence of proteases in all proteinaceous seedlings. [These have also been found in unripe seeds by ZALESKI (1905).] According to the observations of PURIEWITSCH (1897), such an assumption is certainly not necessary in all cases. Whilst only amido-compounds can be recognized in the culture fluid when cotyledons of lupins empty themselves of their own accord, other seedlings treated in a similar way give off into the surrounding fluid either amides in addition to proteid or peptone, or peptone and proteid only. It must be assumed from this that proteid as such can pass through the protoplasm and cell-wall, a possibility which we have not hitherto generally taken into account, but one which, especially after our experience of the ability of fats to penetrate such walls, cannot be regarded as unlikely.

In the decomposition of proteid, sulphates also are set free, as mentioned on p. 139. The globoids give off phosphoric acid (compare ZALESKI, 1902), which may arise also from lecithin. Many seeds contain not inconsiderable quantities of this organic compound, which contains phosphorus but no sulphur, and hence in the process of germination much phosphoric acid may arise from this source. [According to more recent investigations only a *very small part* of the phosphoric acid arises from lecithin; phytin (oxymethylphosphoric acid), on the other hand, appears to be the most important source of this acid. (Compare p. 145). (POSTERNAK, 1903; IWANOFF, 1902).] It is doubtful whether phosphoric acid occurs in seeds in a less complex combination, e. g. as an inorganic salt. The same may be said of other ash constituents; calcium and magnesium, and apparently iron also, are present in the globoids in organic form, but perhaps they may occur in seeds in other forms as well. [POSTERNAK (1905) shows that all the essential materials of the ash are present in the aleurone grain, potassium, silicon, and manganese, as well as those mentioned; whether they are all present in the globoid he does not say.]

Reserve substances are by no means restricted to the storage tissues of *seeds*, on the contrary they are found wherever individual cells or tissues occur independently of *special* assimilatory activity. Next to seeds stand those organs which subserve the purposes of reproduction and multiplication, viz. those endless types of structures which are known as spores and propagative buds, and amongst these must be included the pollen-grains of the flowering plants. The special significance of the reserves in all these cases lies in the fact that they serve to support the new individual in its development until it has reached independence and can live on the products of its own assimilatory activity, or until it has fulfilled its function (pollen). In other cases, the reserves render possible the formation of new vegetative organs in such plants as pass through a resting period during which they rid themselves of all superfluous structures.

This is the case with those perennials which lose all their aerial parts in winter, and in trees which, at least in many cases, cast off their leaves in the winter season. Furthermore, reserves develop in assimilatory organs themselves when the assimilatory products are formed more rapidly than they are used up or removed. In all cases, however, before the reserves are actually employed they must be chemically altered and rendered mobile. This we have yet to consider, although we may dismiss the subject in a few words since the methods employed are fundamentally like those already referred to in seeds.

The reservoirs for reserves in perennials are in the form of aggregations of large-celled storage parenchyma situated in the interior of the plant, and often indicated externally by conspicuous swellings. The storage tissue may occur in the root, in the hypocotyl, in the stem, or in the leaf, and hence we are led, from the morphological point of view, to distinguish such swellings as root tubers, stem tubers and bulbs. In close relation to such storage tissues we find one or more buds which are capable of developing into shoots in the following year. The reserves present in these bodies are on the whole the same as those found in seeds, and consist of nitrogenous and non-nitrogenous organic substances as well as constituents of the ash, which we need not consider further. In one point only seeds differ from subterranean storage organs, viz. that when ripe they are for the most part in a desiccated condition, and the absorption of water is the essential preliminary to their germination. Subterranean reserve organs, on the other hand, always contain a considerable percentage of water; if they be artificially deprived of water to the same extent as seeds are, they would in most cases soon come to grief. It is well known, for instance, that potato tubers are able to develop shoots if not in too dry an atmosphere, owing to their possessing a store of water, and that some bulbs and tubers may give rise to shoots bearing flowers, without any absorption of water being observable. MEDICUS (1803) noted this in the case of *Veltheimia capensis*; HILDEBRAND (1884) drew attention to the same fact in *Oxalis lasiandra*, and recently *Sauromatum guttatum* has been put on the market as a curiosity in consequence of its power of forming flowers in the complete absence of water, after being heated. We may indeed say that *water* itself is, in many subterranean storage regions, a reserve substance.

Amongst *non-nitrogenous reserves*, carbohydrates are entitled to the first place for they are even more abundant in these underground storage organs than they are in seeds. On the other hand, *fats*, so common in seeds, occur but rarely in subterranean storehouses (e. g. *Cyperus esculentus*). The carbohydrate present is very frequently starch, although we often find present in addition, or exclusively, substances which we have not mentioned in speaking of seeds, because they are either absent or are of secondary importance. Such substances are mucilage and varieties of sugar. Mucilage as a reserve may be found abundantly in the tubers of Orchidaceae and in the rhizome of *Symphytum* (compare FRANK, 1866). [Mucilage corresponds to reserve cellulose in these situations and gives, on hydrolysis, mannose and galactose. HÉRISSEY, 1903.] Among the forms of sugar glucoses occasionally occur as reserves, e. g. in the onion. These sugars are capable of being used in the plant for other purposes without further transformation; since, however, their accumulation must obviously cause an increase in osmotic pressure, we may conclude that the plant for the most part unites several molecules of glucose into a molecule of larger size with separation of water. Thus, for example, the transformation of glucose into cane sugar reduces the osmotic pressure by one-half, and it will be further reduced if substances such as inulin are formed, which have a composition similar to that of starch, but which remain in solution in the cell-sap. Inulin occurs as a reserve especially in the Compositae and Campanulaceae; a substance at least very like it in composition is found also in certain Liliaceae. Cane sugar is the dominant reserve in sugar-beet and also in the sugar-cane

(which, however, strictly speaking, should not be referred to here). A more detailed account of the distribution of the different varieties of sugar is as out of place as a complete enumeration of those which have been found. We need only note that cane sugar and inulin undergo alteration during germination, although we might assume a direct conversion from their solubility in water. The alteration once more consists in a hydrolytic decomposition by the agency of enzymes. The enzyme invertase decomposes the cane sugar into equal parts of dextrose and levulose, while inulase transforms the inulin into levulose.

Proteid is always present among the *nitrogenous reserves* of perennial plants, occurring occasionally in the form of crystals (e. g. in the potato); it, however, never forms aleurone grains owing to the large amount of water present. Besides proteid, amido-compounds, such as asparagin, leucin, and tyrosin, also occur, and these, in the majority of cases, may be assumed not to arise from precedent proteid but to be stored as such. In the tubers of a certain variety of potato, SCHULZE (1882) found 56 per cent. of the total nitrogenous substance present consisted of amido-compounds, and only 44 per cent. of proteid. In the sugar-cane SHOREY (1897) found the simplest amino-acid, glycocoll, a substance which has not as yet been shown to occur elsewhere in plants.

The most extensive storage tissue occurs in *trees*, where all the parenchyma cells of the wood, of the cortex, and also of the pith, both in root and stem, are filled with reserves. The central elements of the wood of many trees which gradually pass over into duramen and, on losing their vitality, cease to store reserves, form an exception. Starch is the most generally distributed of all the *non-nitrogenous substances* in such plants, and, owing to the ease with which it can be recognized, its behaviour can be closely followed. Storage of starch begins in May or June, and generally first of all in the cells of the root; later, it appears in the stem, and finally the cells of the branches and finer twigs become filled with it. During the winter it undergoes alteration, completely or partially, enabling it to be translocated to other regions; but in spring, before the buds come out, the same conditions as in autumn are re-established, and in order to render the starch mobile, diastase must be produced in sufficient quantity. [In addition to starch, hemicelluloses also occur as non-nitrogenous reserves in trees; these bodies take the form of thickenings on the walls of the wood fibres or cortical parenchyma, which become again dissolved in spring (LECLERC, 1904; SCHELLENBERG, 1905).] Our knowledge of the *nitrogenous* reserves in trees is much less extensive; in general, they consist of proteids and amides. Proteid in a crystalline form may be demonstrated in some situations, for example in the bud-scales of certain trees, which act a storage organs in the same way as do bulb-scales, and which contain not only proteid but non-nitrogenous substance as well—mostly in the form of reserve cellulose.

The last type of storage organ, the *foliage leaf*, brings us back again to structures with which we are more familiar. We have already studied in detail the synthesis of carbohydrate in these organs and have seen that it is probable that proteid is formed there also—if not exclusively, still in very large quantity. It has also been more than once indicated that fats of value in metabolism are scarcely at all formed in the foliage leaf. [In leaves which last for several years, fats acting as reserves certainly occur, at least in winter, just as in the case of trees (p. 175).] So long as the leaf is growing the products of assimilation are at once made use of, or are so rapidly translocated that an accumulation of them is impossible. In general, the products of assimilation, or more accurately, the surplus over immediate wants, become reserves in the regions of their formation, but they do not remain in this condition for long; most commonly, they again become mobile during the night succeeding their construction and migrate out of the leaf. In the case of starch, such periodic formations and dissolutions have been definitely demonstrated. Phenomena such as these, however, suggest

several new problems, and more especially lead us to inquire whether the dissolution of reserves in the leaf is also effected by means of *enzymes*.

From a comparison of the results of experiments conducted by VINES (1891), JENTYS (1892), and also by BROWN and MORRIS (1893), the occurrence of diastase in the foliage leaf cannot any longer be doubted. Although WORTMANN (1890) arrived at another conclusion, still his failure to find diastase can be readily explained; for diastase, in the first place, is present in the foliage leaf only in small quantity, and further, it cannot be extracted completely by water, considerable further loss taking place during filtration. The chief reason for the apparent absence of diastase lies in the *tannin* so frequently present in the leaf, which by precipitating the diastase makes it inactive. To BROWN and MORRIS we owe convincing proof of the fact, not only that diastase is present in the leaf, but also that it occurs in sufficient quantity to transform into sugar all the starch present there. These authors also showed that different leaves contain the most varied amounts of diastase. For this purpose they estimated the amount of maltose which was produced from so-called soluble starch in forty-eight hours by the action of an extract of 10 g. of dried and powdered plant substance, and found that 10 g. of malt gave 634 g. of maltose, 10 g. of the leaf of *Pisum* gave 240 g., 10 g. of *Lathyrus* leaf gave 100 g., 10 g. of *Tropaeolum* leaf gave 4–10 g., and of *Hydrocharis* leaf, 0·3 g.

In comparison with malt the amount of diastase present in leaves is generally small, although many leaves have large supplies. Definite proportions evidently exist between the starch contents of a leaf and diastase, since the Leguminosae which have been examined and which are next after malt in diastatic power, are at the same time very rich in starch; on the other hand it must be noted that not all the numbers obtained can be accepted as an exact measurement of the diastatic capabilities of the leaves concerned without further confirmation; more especially we must note how the very inactive leaf of *Hydrocharis* owes its place at the end of the series chiefly to the large amount of tannin which it contains, which, as we have already said, inhibits the action of diastase. Among external factors, so far as they have not been already mentioned, we must note light, which has an inhibitory action on diastase (GREEN, 1897), so that more starch is altered into sugar at night than by day. EMMERLING (1901) was unable, however, to confirm this observation. Again the increased activity of diastase in darkness, due to carbon-dioxide (MOHR, 1902), must tend to make the amount of sugar formed at night greater, since at night the leaf contains much more carbon-dioxide than by day. How far our previous conclusions as to the amount of material formed during the process of assimilation by day are actually affected by this fact we cannot at present say; it will be remembered that we assumed that quite as much carbohydrate was translocated by day as by night. Perhaps also SACHS'S estimates (p. 114) are too high.

As we have seen, diastase, generally speaking, transforms starch into maltose, i.e. a reducing disaccharide, nearly related to cane sugar. The maltose is by hydrolysis broken up into two molecules of dextrose, while cane sugar is decomposed into a molecule of dextrose and a molecule of levulose. Since cane sugar has been shown to be without doubt a product of carbon assimilation, at least in certain plants, but that maltose is a product of the decomposition of starch, it comes to be a question whether these disaccharides undergo further conversion *as such* or whether they must be *hydrolysed* first in the manner described. A final decision on this question has not yet been reached. Reference may first be made to the germination of beet, where there is no doubt that cane sugar is transformed into invert sugar. That the presence of invertase is necessary for this decomposition has been established with sufficient certainty in various plant organs (GREEN, 1901), and BROWN and MORRIS have proved its presence in the leaves of *Tropaeolum*, KOSMANN in buds of trees, O'SULLIVAN

in barley seedlings, VAN TIEGHEM and GREEN in pollen-grains; in a word the wide distribution of invertase can scarcely be called in question. At the same time we do not wish to imply that cane sugar cannot be *directly* converted in some other way. Still more difficult is the case of maltose. We noted (p. 150) that, according to BEIJERINCK, dextrose could be formed from starch by means of a special diastase, and this effect might well be produced by the action of glucase, a member of the diastase group. Such a ferment has been shown to occur in yeast, but not much is known as to its occurrence in the higher plants. We must also remember in what has yet to be said that, besides dextrose and levulose, saccharose and maltose may occur as migratory carbohydrates, not to speak of glactose and mannose, about which as yet very little is known.

Our imperfect acquaintance with the mode of formation of *nitrogenous* products of assimilation in the foliage leaf has already been frequently noted. Proteids and amides have frequently been found in the leaf; the amides are capable of translocation without further alteration, but the proteids must, at least in some cases, undergo decomposition first. That such a decomposition of proteid is probable may be deduced from the fact that an accumulation of amides has been observed in such organs as have been kept in the dark (BORODIN, 1878). The formation of amides is not influenced by darkening, but the reconstruction of proteid from them is prevented, and hence their accumulation is explained. We have indirect evidence also of the decomposition of proteid. The occurrence of a tryptic ferment in a number of succulent organs has been demonstrated, and this ferment is capable of bringing into solution the proteid formed in the course of assimilation, at all events there is no other purpose known which it could fulfil. The most thoroughly investigated is a trypsin found in the fruit of the pineapple, and which CHITTENDEN (compare GREEN, 1901, 198) has termed bromelin. It acts very energetically on fibrin and on egg albumen, and gives as products of its action, peptone, leucin and tyrosin. Were this enzyme limited in its occurrence to this fruit it would be of little interest to us here, since we are investigating the enzymes present in the foliage leaf, but another proteid-dissolving enzyme, papain, known at first only in the fruit of the papaw tree, has been shown by WURTZ (1879) to occur also in leaves, so that we may believe that bromelin also may occur in the vegetative organs of the pineapple. Tryptic enzymes have also been obtained by MARCANO from the expressed sap of the leaves of many species of *Agave*, by BOUCHUT and HANSEN from the sap of the fig (*Ficus carica*), and also by DACCOMO and TOMMASI from *Anagallis arvensis* (for literature, see GREEN, 1901, 212; compare also FERMI and BUSCAGLIONI). In comparison with the doubtless quite general distribution of diastase, evidence of the occurrence of proteases is as yet very scanty, and it would be rash on our part to conclude from such evidence *only* that these enzymes were at all generally dispersed.

Bibliography to Lecture XIII.

BORODIN. 1878. Bot. Ztg. 36, 801.
BROWN and MORRIS. 1890. Journal Chem. Soc. Trans. 57, 458.
BROWN and MORRIS. 1893. Ibid. 63, 604.
BUTKEWITSCH. 1900. Ber. d. bot. Gesell. 18, 185 and 358.
EMMERLING. 1901. Ber. d. chem. Gesell. 34, 3810.
FERMI and BUSCAGLIONI. 1899. Centrbl. Bakt. II, 5, 63.
FLEISSIG. 1900. Physiol. Bedeutg. d. ölartigen Einschl. in *Vaucheria*. Diss. Basel.
FRANK. 1866. Jahrb. f. wiss. Bot. 5, 161.
GODLEWSKI. 1877. Flora, 60, 215.
GREEN. 1887. Phil. Trans. 178 B, 39.
GREEN. 1890. Proc. Roy. Soc. 48, 370.
GREEN. 1897. Phil. Trans. 188 B, 167.

Green. 1901. Die Enzyme. German edition by Windisch. Berlin.
Griessmayer. 1897. Die Proteide d. Getreidearten. Heidelberg.
Grübler. 1881. Journ. f. prakt. Chem. 131, 97.
[Hérissey. 1903. Rev. d. Bot. 15, 345.]
Hildebrand. 1884. Lebensverhältnisse d. Oxalisarten. Jena.
Holle. 1877. Flora, 60, 113.
[Iwanoff. 1902. Ber. d. bot. Gesell. 20, 366.]
Jentys. 1892. Bullet. Acad. d. Cracovie.
König. 1882. Chem. Zusammensetzung d. menschl. Nahrungsmittel. Berlin.
[Leclerc du Sablon. 1905. Rev. d. Bot. 16, 341.]
Medicus. 1803. Pflanzenphysiol. Abhandlungen, 2, 140.
Mohr. 1902. Centrbl. Bakt. II. Abt. 8, 601.
Neumeister. 1894. Zeitschr. f. Biol. 30, 447.
Newcombe. 1899. Annals of Botany, 13, 49.
[Posternak, 1903. Compt. rend. 137, 202, and 1905, 140, 322.]
Puriewitsch. 1897. Jahrb. f. wiss. Bot. 31, 1.
Sachs. 1859. Bot. Ztg. 17, 177 (Ges. Abh., 1, 557).
[Schellenberg. 1905. Ber. d. bot. Gesell. 23, 36.]
Schmidt. 1891. Flora, 74, 300.
Schmiedeberg. 1877. Zeit. f. physiol. Chem. 1, 205.
Schröder. 1901. Bot. Centrbl. Beihefte, 10, 122.
Schulze. 1882. Versuchsstationen, 27, 357.
Schulze. 1890–2. Zeit. f. physiol. Chem. 14, 227; 16, 387.
Shorey. 1897. Cited in Revue gén. de Bot. 1902; 14, 283.
Sigmund. 1890–92. Cited by Green, 1901.
Tschirch. 1900. Ber. d. pharm. Gesell. 10, 214.
Vines. 1891. Annals of Botany, 5, 409.
[Vines. 1905. Ibid. 19, 171.]
Weyl. 1877. Zeit. f. physiol. Chem. 1, 72.
Wortmann. 1890. Bot. Ztg. 48, 581.
Wurtz. 1879. Compt. rend. 89, 425.
Zaleski. 1902. Ber. d. bot. Gesell. 20, 426.
[Zaleski. 1905. Ibid. 23, 133.]

LECTURE XIV

THE CONVERSION OF THE PRODUCTS OF ASSIMILATION. III

THE TRANSLOCATION AND TRANSFORMATION OF DISSOLVED RESERVES

The reserves accumulated in storage organs become at definite times *mobile*, that is to say, they are transformed from an insoluble and non-diffusible into a soluble and diffusible state. The object of this transformation is to permit of these substances migrating from cell to cell, and such translocation of material from the storage regions to the places where they are used up is a phenomenon of wide occurrence in the plant. It is very easy to prove the migration of *carbohydrates* out of the foliage leaf, and much research has been carried out on this subject. One can often observe that a leaf which is full of starch in the evening has become quite empty of starch next morning, after a warm night, if it remains attached to the plant, but that if it be cut off, the amount of carbohydrate suffers but little change during the night. We may, therefore, conclude that a migration of carbohydrate takes place out of the normal leaf in the dark. This migration does not cease, however, by day.

It is more difficult to prove the migration of *nitrogenous substances* from the foliage leaf. Exact research on this subject is as yet not forthcoming. Kosutany, it is true (1897), has made careful comparative researches on the amount of nitrogenous substance present in vine leaves in the afternoon and in the morning before sunrise, but unfortunately he based his calculations on

corresponding *dry weights* and not on similar *leaf areas*. In 100 g. of dry substance he found as follows :—

	Total Nitrogenous matter.	Proteid.	Non-proteid.
In the afternoon	3·537	3·199	0·338
In the early morning	3·621	3·385	0·236

He concluded from these numbers that *during the night* a *formation* of proteid from non-proteid (nitric acid and amides) took place, and that the total amount of nitrogenous material *increased*. This conclusion does not appear to us to be sound. We will attempt to reduce his determinations to a calculation of similar leaf surfaces, using as a basis the values which SACHS obtained for the loss in weight during the night of the leaves of *Helianthus* and *Cucurbita* :—

	Evening.	Morning.
1 sq. m. *Helianthus* (dry) weighs	80·44 g.	70·80 g.
1 sq. m. *Cucurbita* (dry) weighs	59·92 g.	51·22 g.
Total	140·36 g.	122·02 g.
On an average one sq. m. of dry leaf substance	70·00 g.	61·00 g.

If 70 g. of dry leaf substance in the evening represent the same leaf area as 61 g. in the morning, then 100 g. in the evening will be represented by 87 g. in the morning ; in other words, a definite area of leaf blade loses 13 per cent. of its dry weight during the night by translocation of the products of assimilation. If we assume for the vine in KOSUTANY'S researches a loss of only 10 per cent., to use round numbers, then 100 g. in the afternoon would correspond to 90 g. in the morning. We might then rewrite KOSUTANY'S tables, for similar leaf surfaces, in the following way :—

	Total Nitrogenous matter.	Proteid.	Non-proteid.
A definite leaf area contains in the afternoon	3·539	3·199	0·338
The same leaf area contains in the morning	3·259	3·047	0·212

From such a calculation it would be possible to deduce conclusions as to the *migration* of nitrogenous substance during the night, and not as to its *increase* in the individual leaf. An experimental confirmation of our argument, resting as it does on a somewhat insecure basis, would be certainly of value.

We have to note as well that, in addition to this *daily* translocation, *another* transference of nutritive material occasionally takes place from the leaf. In the first place, the leaves of evergreens frequently act as storehouses of reserve and empty themselves in spring, just as do the cotyledons of a seedling ; before it dies, however, certain bodies migrate out of the leaf back into the permanent living parts of the plant. This transference of material was for long greatly overestimated, until WEHMER (1892) pointed out that it was in no way substantiated by facts. More recently RAMANN (1898) has demonstrated in forest trees, and FRUWIRTH and ZIELSTORFF (1901) in hops, that, as a matter of fact, nitrogen, phosphoric acid, and potassium do migrate from the leaves in autumn. This translocation can scarcely, however, be considered of much importance ; this brief reference to the subject must therefore suffice.

We need not pause here to give in detail the evidence for the translocation of materials from other storehouses ; we have had other opportunities of considering the subject, at least in part, and, in the course of our further investigation, we will return to the subject when we study the *causes* of translocation in greater detail and the *path* by which the translocation products move.

In the first place, we may consider certain purely *physical* causes of translocation. We have already, in speaking of germination, and in the discussion of the researches of HANSTEEN and PURIEWITSCH, drawn attention to one fundamental principle of every translocation, viz. diffusion. It is quite immaterial whether the diffusion takes place from one cell to another or from the cell to the exterior ; all that is necessary for the initiation of diffusion is that the

solutions of a substance in two regions shall have different degrees of concentration. Thus we saw that when the endosperms of grasses were immersed in a large quantity of water the cells were gradually emptied, while a small amount of water rapidly came to contain so much sugar that diffusion could no longer take place. When the migration of the sugar formed from the starch ceases hydrolysis of the starch also comes to an end and the endosperm-cells remain full. It was further noted that the emptying of the endosperm was stopped more rapidly by immersing the storage region in a solution of sugar than by employing a *small* quantity of *water*. The same method of emptying reserve stores was also employed by PURIEWITSCH in the case of isolated cotyledons, root tubers, rhizomes, bulbs, and branches. [Compare WÄCHTER, 1905.] It might also, perhaps, be possible by appropriate methods to induce an emptying of an isolated foliage-leaf filled with products of assimilation. In the case of the storehouses mentioned, another highly important method of investigation into the migration of reserves may be successfully carried out, for which endosperm is obviously not suited, owing to the fact that it dies off after the emptying of its cells. As already noted we may prevent translocation by means of a sugar solution of appropriate concentration, but if the concentration be increased one finds the opposite process taking place, for the sugar enters the storage tissue and there forms starch. This phenomenon is identical with that recorded at p. 112, where it was seen that the formation of starch took place in the foliage leaf when sugar was supplied from without. In this experiment there are two points of interest for us which we have not previously paid attention to. In the first place, it shows us that the direction of the nutritive stream is determined by the degree of concentration at two different points. Whether an outflow or an inflow of materials takes place in any cell depends, at least to a certain degree, on its surroundings; both an outflow and an inflow may take place at the same time if the contents of the neighbouring cells on one side show a higher, on the other a lower, degree of concentration of the nutrient in question. Just as in the case of the single cell, so also a tissue situated between two other tissues with different sugar concentrations will permit sugar to stream through so long as this difference is maintained. In other words, physical conditions well known and easily understood govern the situation in this case. The refilling of empty storage regions is instructive from a second point of view. It shows that a continuous removal of the sugar formed, using a very large amount of water in the emptying experiments, is by no means always necessary for the maintenance of the diffusion current, and that the translocation may be replaced by storage and transformation. In fact, the movement of materials into an empty cotyledon would soon stop, were it not that the entering sugar is changed into starch, and thus room is made for new supplies. It need scarcely be mentioned that the principle of diffusion already mentioned, i. e. the maintenance of the flow by translocation or storage, is not limited to the case of sugar and starch, where it can be conveniently demonstrated, but that it applies to all other migratory substances. The principle is by no means a new one; on the contrary, it has been discussed in detail in relation to the osmotic characters of the cell, although its importance may be again emphasized here.

The diffusion flow is, however, not the only important factor in the migration of materials; the permeability of the protoplasm is of equal importance. It would be unfortunate for the plant if its cells permitted all the reserves they collected to diffuse outwards in the way they do in PURIEWITSCH'S researches on storehouses of reserve. A rapid streaming of materials would then take place towards the roots, and from them into the soil; the existence of the plant would then become impossible. If the plant is not to lose all its reserves by diffusion, the external walls, which are in close relation to water, must not permit of the passage of the reserves through them. In all probability this

impermeability lies in roots and submerged plants in the *external layer of the protoplasm*, whilst in aerial parts the impermeable cuticle prevents the washing out of reserves by rain. It is very desirable that research should be undertaken to determine more accurately than has hitherto been done, whether permeability and impermeability are constant characteristics of the protoplasm of a definite cell, or whether, as is more probable, the characters of the protoplasm are capable of variation according to the demands made upon it. [Compare NATHANSOHN, 1904.] There is quite a number of phenomena which could, perhaps, be interpreted in the former sense, but which might just as well be dependent on some more complex influence of the protoplasm. At all events, the facts cannot be explained merely by taking into account the principle of diffusion, for we have to deal, not with a simple osmotic apparatus, but with a perpetually changing organism. The facts to which we allude have been already mentioned (p. 156). If the emptying of the storehouses of reserve were governed by purely physical factors, then the artificial emptying of the endosperm in PURIEWITSCH'S experiments could only have been arrested by such bodies as are formed in the hydrolysis of reserves; as a matter of fact, substances also act in an inhibitory way, which are not subject to the laws of diffusion; oxygen and chloroform especially have an influence on the emptying process. Thus CZAPEK (1897), experimenting with leaf stalks, was able to show that killing or stupefying with chloroform retarded the translocation of products of assimilation, whilst an atmosphere of carbon-dioxide did not affect it. WORTMANN (Bot. Ztg. 1890), however, has arrived at an exactly opposite conclusion in experimenting with carbon-dioxide. The possibility that phenomena of this kind depend on variations in the quality of the plasmatic layer must be conceded, but we must not forget the far-reaching influence of the whole protoplasmic machinery of the cell; translocation is thus by no means so simple a process as it has been hitherto considered.

We are driven to the same conclusion for other reasons. Diffusion *works much too slowly* to accomplish *by itself* the transportation of the materials in the plant. DE VRIES (1885) has shown that, from STEPHAN'S calculations, a milligram of sodium chloride, one of the most rapidly diffusible salts, takes 319 days, almost a year, in order to migrate from a 10 per cent. solution into water a metre distant. The same result would be obtained in the case of cane sugar in two and a-half years, and in the case of proteid in fourteen years. The slowness of diffusion may be demonstrated very clearly in the following way:—Take a long glass tube, closed at one end, and place in it a solid coloured salt such as copper sulphate, and then fill the tube with water or a not too concentrated solution of gelatine. The rapidity of diffusion is quite as great in the latter as in water. After a week, the copper sulphate will have reached a height of 5 cm., after five weeks, 13 cm., after three months, 20 cm. If the tube filled with stiff gelatine be inverted it will be seen that *weight* has no influence on diffusion, a matter of importance in determining the translocation of materials in the plant.

This experiment leaves us in no doubt that simple diffusion cannot account for the translocation of such quantities of material as migrate from the foliage leaf in the course of a single night. Some means of accelerating the movement must exist. One factor is the streaming movement taking place within the cell, thus bringing about a mechanical mixing of the materials. Such rapid movements result from unequal heating of different parts of the cell, perhaps, also in consequence of electric currents which are widely diffused in the plant, and also in consequence of protoplasmic movement. Although in this way, in a very short time, a uniform degree of concentration of a certain substance may be reached in a single cell, still diffusion is needed to account for the passage from cell to cell and for the penetration of the cell-wall and the two layers of protoplasm which lie against it. It must not be supposed that the cell-wall places

greater difficulties in the way of the diffusion of substances soluble in water. As a matter of fact, one sees that the aniline dyes mentioned previously pass very rapidly through the outer walls of a cell of an alga, and the cell-walls lying between the parenchyma cells may be compared in their characters with such an algal cell. In the walls of all cells pits occur with great regularity, that is to say, places where the wall remains thin on both sides, and these may serve to shorten the path which the diffusing particles have to take through the membrane. This fact led to the belief that the cell-wall was more difficult to penetrate than the protoplasm. Since we know that the pits are pierced by numerous fine pores, and that by their means not only is the protoplasm of one cell in continuity with that of its neighbours, but that in this way the protoplasts of the whole plant form one connected system, we must view the pits, as agents for permitting translocation of materials, from an entirely different point of view. It might be imagined in the first place that formed particles of plasma or entire starch grains might be squeezed through these minute canals; as a matter of fact, MIEHE (1901) and KÖRNICKE (1901) have seen even nuclei pass through the membrane, doubtless by way of these protoplasmic bridges, but such migrations, owing to the minuteness of the pores, can be possible only under high unilateral pressures, such as scarcely ever occur in nature. PFEFFER (1892), in investigations specially devised for the purpose, was unable to observe any passage of protoplasm through the pores in the pit-closing membrane. Although the significance of the protoplasmic bridges as agents in the transport of materials in mass is, to say the least, doubtful, still they are obviously of great service in diffusion movements. We may assume that the protoplasm of each bridge consists of the outer plasmatic layer and the inner plasma, so that although the external layer is impermeable, as it is in the cell, still the materials may be able to diffuse through the inner plasma. It is true the canals are *very narrow*, but they are, on the other hand, very numerous and very short, and from BROWN'S work (1900) (compare p. 121) we know that, with a suitable arrangement and size of the pores, diffusion can be quite as great as though the entire pit-closing membrane were absorbed.

According to these determinations it appears that a transference of materials will succeed more easily in long cells in which few partition walls have to be passed through than in short ones. This leads us to consider somewhat more closely the tissues which subserve translocation of materials in the plant. Each normal parenchyma cell can fulfil this function and, as a matter of fact, we find in certain regions that these cells are the only ones carrying out this function. In endosperm parenchyma cells alone are present and other elements are absent from all growing points. But it must be noted that growing points exhibit only very slow growth changes and hence a rapid transference of materials is not required. Behind these, where active growth is taking place, tissue differentiation is more manifest, and there we find cells, which obviously are adapted specially to the transport of material, more accurately, of mobile organic substances. These are the sieve-tubes, which, not only from their great length, but also from the partial absorption of their transverse walls by sieve-pores, are peculiarly suited to this purpose. They form long strands lying close to the vascular cords and constitute along with these the 'vascular bundle'. Let us consider, as an illustration, the emptying of a leaf that has been assimilating all day, and inquire as to the part played by the sieve-tubes in the process. SCHIMPER (1885) made an interesting investigation on *Plantago*, in which type it is possible to remove the vascular bundle from the leaf stalk without causing excessive injury, the leaf meanwhile remaining attached to the stem. SCHIMPER found that a leaf so treated could transfer its starch into the stem in the dark, and he imagined he had discovered in the elongated cells which surround the vascular bundle the so-called 'bundle-sheath', the conducting organs for sugar translocation. On the other hand, CZAPEK (1897) pointed out that, although

there was no reason to doubt the conducting power of these cells, still they were not sufficiently extensive to carry out the transport of the whole of the materials; he held that this was the chief task of the sieve-tubes. Although CZAPEK was unable to advance any definite proof of his view, nevertheless it would seem highly probable that this is the case. He made incisions into the leaf-stalk of *Vitis* on a summer evening, cutting through half of the vascular bundle, and found in the morning that the part of the lamina which was thus partially isolated was still filled with starch, but that the other portion was empty. Since, in this experiment, not only the sieve-tubes but also the bundle sheath was cut through, one cannot draw any conclusion against SCHIMPER'S view; we can only conclude that the general fundamental parenchyma of the petiole is not sufficient for the translocation of the products of assimilation, and further that this transference cannot be effected in a transverse direction, and hence that elongated elements are necessary. The same conclusion is arrived at from experiments in ringing trees. If from an unbranched stem we remove a ring of cortex, right into the wood, the part of the tree situated below the region of ringing is not filled with starch, whilst the part above the ringing accumulates it abundantly. If a narrow bridge of cortex be retained, connecting the upper with the lower part of the tree, the sugar flows backwards but spreads laterally very slowly. If the bridge be in the form of a step (⌙) no backward passage of the products of assimilation takes place, since the horizontal part of the bridge does not conduct them. The wood region in the tree is unable to carry the products of assimilation backwards, and, in the cortex, conduction takes place only in the longitudinal direction. This may depend on the special capacity of the cortical parenchyma; it is more likely, however, that it is the *sieve-tubes* that are the organs of conduction. [HABERLANDT (1904) has, however, advanced arguments against this view.]

If we accept CZAPEK'S hypothesis as to the function of the sieve-tubes we obtain the following picture of the translocation of carbohydrates from the organs of assimilation. The sugar arising from the transformation of starch eventually reaches the sieve-tubes after migrating through the assimilating cells and the bundle sheath. G. KRAUS (compare PFEFFER, Phys. I, 592) found 38 per cent. of the dry weight of sieve-tubes consisted of soluble carbohydrates. In the sieve-tubes it is capable, by mechanical means, by streaming of all kinds, of travelling rapidly for long distances. The sieve-tubes may, under certain conditions, form a strand several centimetres or even decimetres in length, which operates just like a single cell; by diffusion it receives the sugar in at the upper end and gives it off at the lower. Movement in the intermediate region seems not to be effected by protoplasmic streaming, since such streaming appears to be absent in sieve-tubes (STRASBURGER, 1891, 363); still we may conceive of a movement in mass, caused by varying osmotic pressures in the surrounding parenchyma. An exudation of contents due to pressure of neighbouring cells may indeed be observed when sieve-tubes are cut across. We must not, however, suppose the function of a phloem strand to be merely to serve as a means of communication between two regions some distance apart, as though these were in connexion by means of a glass tube. On the contrary, the sieve-tubes during their entire course are in lateral connexion with the phloem parenchyma and give over to them all surplus carbohydrate, and these, owing to vigorous starch formation, are always ready to absorb new materials. The parenchyma which lies in contiguity with the sieve-tube, acts as a storage tissue, and, indeed, as in the case of trees, in a double sense. In the first place, certain reserves are deposited in them, as in the parenchyma of the medullary rays, of the cortex, and of the wood, for next spring; but starch, not only in the stem, but in every petiole, is also deposited in the phloem parenchyma as so-called temporary reserve, that is to say, the surplus inflowing sugar wanders out of the sieve-tubes and from time to time may

be transformed, when a direct supply from the leaf ceases. Such transitory formation of starch always accompanies a translocation of sugar, whether it takes place for long distances in sieve-tubes or for short distances in parenchyma. According to statements already made this formation of starch is easily understood, since it serves the purpose of maintaining the degree of concentration necessary for diffusion.

If the circulation of carbohydrates in sieve-tubes be interrupted, these elements are found to be in general the conducting organs of migratory organic substances. The sieve-tubes have been for long claimed to subserve the carriage of proteid, and the open passage from segment to segment has been referred to as especially of importance, rendering possible the rapid movement of a substance in itself diffusible with difficulty. We need not enter further into a consideration of the translocation of proteid and its decomposition products; what little is known proves that conditions similar to those governing the translocation of carbohydrates prevail here also. It must be noted, however, that materials of the ash also, sometimes as such, at other times in an organic form, must be transported by the same path as proteid and sugar, after they have ascended in the transpiration current from the root and been partly altered into some other form. It is more than possible that sieve-tubes are aided in the performance of their functions by *laticiferous tubes* (compare HABERLANDT, 1883, SCHIMPER, 1885, GAUCHER, 1900). [According to KNIEP (1905), this does not as yet rest on sufficiently secure evidence.]

There remains for us to inquire into a phenomena which is especially exhibited by trees. When in springtime starch is dissolved, the sugar, in order to reach the seat of metabolism has often to travel from a few to more than 100 metres. Hence it may be concluded that it travels by another path and not, or not entirely, by the sieve-tubes, but follows the water-stream in the vessels, just as the salts of the soil do after being absorbed by the root. This conclusion is arrived at from experiments on ringing, as TH. HARTIG has already shown (1858). While, as above noted, such a cortical ringing prevents the accumulation of starch in the basal part of the stem, if the operation be performed *after* the storage of starch in autumn, the whole of it disappears in the *following spring* out of the wood and cortex of the stem base. After A. FISCHER'S (1890) researches there can be no doubt that the glucose is transferred to the opening leaf-buds by the wood and especially by the vessels. And since the transpiration current is effected for many metres while diffusion perhaps is active for only millimetres or microns, one can comprehend the advantage which a plant obtains by this arrangement. Again, since in the sap excreted by a tree in the process of bleeding both amides and proteids have been found, one may well assume that nitrogenous materials travel the same way as do carbohydrates.

TH. HARTIG and also A. FISCHER (1890) and STRASBURGER (1891) have gone further in this respect. They affirm that in trees the upward movement of carbohydrates in spring takes place *exclusively* in the wood, and that only a *downward movement* can take place in the cortex. The reasons advanced in support of this view do not appear to us to be quite sound, and it may be that fresh experiments may show that the phloem also is capable of transporting mobile reserves. This would appear all the more probable since in herbaceous and shrubby plants the vessels are said never to be called into service for the upward transport of reserves, and such a fundamental difference between woody and herbaceous parts would scarcely be intelligible.

The destinations of the migratory materials are always those regions of the plant where materials are being actively used up. The more rapidly the alteration of these materials takes place in the regions of activity the greater the diffusion between the two termini of the movement and the more rapid the movement itself. The dissolution of the reserves will also be accelerated when the migration of the dissolved substances takes place rapidly. In nature there exist

quite definite regions of transformation and others which might be considered as supplying their wants. The *demand* for materials is most prominent in all growing points. In these regions, it is true, no great quantity of materials is called for at any one time, but, since a *continual* formation of new cells takes place in these places, substances needed for the formation of cell-walls, of protoplasm, and of osmotic substances are required almost the whole year round. In a tree, as soon as the *extension* of the shoots of the *present year* is completed, frequently rapidly and always at the expense of last year's materials, early in the year the *primordia* of the *next year's* buds are laid down, the development of which slowly progresses during the winter. Besides, the cambium is also active, and by its constant production of wood and bast, demands a continuous supply of nutritive materials. After the formation of the flowers comes the construction of fruit and seed, and finally the accumulation of stores in the root and stem, beginning at the base and gradually extending upwards. In all cases we recognize as migratory materials the substances so often mentioned, sugar, proteid, and amides, and further, we see that reserves are formed from them, either temporary in their nature or destined to remain quiescent for longer periods. Trees are distinguished from annuals inasmuch as the latter store reserves permanently in their seeds only, while the distinction between trees and perennial herbaceous plants lies in this, that the latter deposit their reserves not in aerial but in subterranean storehouses.

A transference of material takes place normally from all storehouses of reserve, but the plant is, however, able under abnormal conditions to permit of consumption and transference also taking place in other than the normal situations. For example, if we remove the growing points and thus render their development impossible, other appropriate organs may become centres of consumption (compare VÖCHTING'S experiments, Lecture XXVI), and if we allow the growing points to develop with insufficient food supplies, materials are drawn from older parts of the plant and in such quantities that these older organs die off. In cultures carried out in darkness one often notices apical growth proceeding at the cost of the older and moribund leaves.

We have still, in conclusion, and as an appendix to our treatment of the subject of alteration and migration of materials, to note the changes which these moving plasta undergo when they reach their destinations. These changes are most varied, especially when the plasta are altered into constructive materials. We have to compare only such relatively simple migratory bodies as soluble carbohydrates, amides, and minerals with the complicated structure of the cells made from them. As to these metamorphoses of materials we are still very much in the dark. The processes by which reserves are formed from translocatory materials are better understood, since these become transformed, on the whole, into the same bodies as those from which they were derived. Here, also, however, we come face to face with many debatable points. Although, for example, starch or reserve cellulose is formed from glucose we are ignorant of the immediate conditions of the transformation; we can only say that the changes are not very extensive, and it is only a question of time before we shall be acquainted with all the chemical details of the process. The matter is not nearly so simple with proteid. This substance, as we have seen, breaks down into bodies whose constitution is very different from its own. If germination takes place in light no noticeable accumulation of amides takes place, because they are at once retransformed into proteids at the regions where consumption is going on. But if we allow the seeds to germinate in the dark these bodies accumulate in such quantities that we can demonstrate their crystals with the greatest readiness under the microscope, after precipitation with alcohol. Obviously the conditions for the reformation of proteid are not fulfilled in darkness, and hence a culture in the dark is always employed when it is desired to obtain amide bodies in large

quantity. If we now compare the amides appearing in a darkened seedling with those which may be obtained from the decomposition of proteid outside the plant by the aid of acids or enzymes, we find them to exhibit several remarkable differences. In the first place we generally find an abundance of asparagin and glutamin in the plant, whilst the related aspartic and glutaminic acids appear outside. Then again the proportional amounts of the several amino-acids in the plant and outside it are by no means the same ; in the plant, *one* is generally predominant, and the greatest differences present themselves in different plants in this respect. Thus we find in the seedlings of Leguminosae and Gramineae that *asparagin* is especially abundant, whilst in Cruciferae, *Ricinus*, and *Cucurbita*, *glutamin*, and in Coniferae, *arginin* is the dominant compound. These differences are not to be explained by assuming a different composition in the reserve proteid of the seeds concerned; since in the individual species one does not find as a rule the substances under discussion always in the same relation. E. SCHULZE (1898), who has gone most deeply into these questions, formulates the hypothesis that the same decomposition products arise from proteid in the plant and apart from it, but that in the plant a *further alteration* takes place which affects the individual products of the hydrolytic decomposition in varying degree. Changes in the composition of the mixture of organic nitrogenous bodies can be determined directly by analysis. This is apparent in a comparison, for example, of an analysis of pea-seedlings one week old with those three weeks old :—

	Leucin.	Tyrosin.	Arginin.	Asparagin.
1 week	abundant	little	present	absent
3 weeks	much less	absent	almost absent	very abundant

Further, SCHULZE was able to demonstrate the presence of arginin and amido-acids only in the cotyledons of the lupin, but he could find no asparagin, while this latter substance was present in the stem of the seedling ; similarly, the cotyledons of the cucumber contained no glutamin although that substance collects abundantly in the stem. Finally, it would appear from quantitative analyses that the occurrence of asparagin goes hand in hand, not with the disappearance of proteid, but with that of amino-acids.

SCHULZE assumed that the amino-acids first arising from the proteid substances, in addition to which perhaps also primarily asparagin and glutamin may arise, break down further into ammonia, and from this, in presence of a suitable carbohydrate—perhaps glucose—asparagin and glutamin are constructed. These amides would be thus, not the main products of decomposition, but rather the first stages in a higher synthesis, and their formation from this point of view is not inconceivable. According to HANSTEEN'S experiments the amino-acids appear, so far as they have been investigated, much less adapted for the formation of proteid than ammonia or the two amides above mentioned ; an accumulation of ammonia would be a disadvantage, however, because that substance, which can be detected only in traces, readily acts as a poison in larger quantities. This hypothesis of SCHULZE, which has been recently supported by BALICKA-IWANOWSKA (1903) [and also by PRIANISCHNIKOW, 1904, and earlier], appears to us to explain best the facts known to us at the present time in this difficult region of investigation ; much still remains to be done, however, quite apart from the fact that the mode of origin of asparagin from ammonia and glucose in a purely chemical manner cannot be discussed at all at present.

A new difficulty presents itself when we attempt to investigate how proteid arises from amino-acids or amides. It cannot be doubted that the process takes place in light, and PFEFFER (1873) has further given proof that light acts indirectly in the process. BALICKA-IWANOWSKA (1903), however, shows that in all likelihood light has also a *direct* influence. Illumination in conjunction with an atmosphere free from carbon-dioxide does not result in the disappearance

of such amides; certain products arising during carbon assimilation are necessary for proteid formation. We must again take into consideration the carbohydrates in this relation, all the more so since if these are supplied in sufficient quantity proteid reformation can take place in the dark (compare p. 144). The establishment of this fact is far, however, from solving the chemical problem of the synthesis of proteid from glucose and asparagin. The glucose at all events must undergo a fundamental change during this process, since it is inconceivable that it can be introduced into the proteid molecule exclusively as a carbohydrate group; indeed, it is by no means certain that such a group occurs in vegetable proteid. Since, however, in animals carbohydrates can be formed from proteid, we must admit the reverse process to be possible in the plant.

In addition to the regeneration of proteid out of the products of its decomposition we must glance finally at the construction of fats, which we have seen in our last lecture to be present as a reserve material in seeds. Fat also occurs in the vegetative organs, and, according to certain authorities, it may be supposed to travel in these organs either as fat or, after preliminary decomposition into glycerine and a fatty acid. No one can believe, however, that the entire mass of fat occurring in a seed could have entered it in that form. On the other hand, the same materials may be seen travelling to oily seeds as to those poor in oil, i. e. carbohydrate or, e. g., *mannite* in the olive, a substance which takes the place of carbohydrate there. Again, in all oily seeds (PFEFFER, 1872) in the young condition large quantities of starch occur, which, when the seeds are ripe, is replaced by a fatty oil. This does not appear to be effected by the respiration of starch and its change into water and carbon-dioxide while fat wanders in to take its place; on the other hand, the fat must be derived *from the starch*, for one can demonstrate its appearance in isolated unripe seeds into which no entry of fat is possible. Just as we saw earlier that an alteration of fat into carbohydrate took place during the germination of seeds, so now we may note that in ripe seeds starch is changed into fat. From the chemical point of view this change is extraordinarily difficult to appreciate, i. e. the origin of a substance poor in oxygen from one relatively rich in that element. A chemical reaction of this type has not as yet been observed externally to the cell. We must therefore content ourselves for the present with the observation of the fact without being able to enter more deeply into the meaning of the phenomenon. It must be noted, however, that the alteration of starch into fat is not limited to seeds. In trees also starch is, during winter, at least partly, altered into fat, and in spring starch is again reformed from fat. [According to NIKLEWSKI (1905), the relationship between sugar and fat cannot be explained from the chemical point of view and physiologically also it is very doubtful.] Both these processes depend in a variable manner on temperature (A. FISCHER, 1890); low temperatures tend to induce the formation of fat, high temperatures, starch. Hence one can in the middle of winter bring about a reformation of starch in amputated twigs by bringing them into a warm room. The *significance* of this phenomenon is still a great puzzle, and its physiological reasons are also but little understood. An increase in dissolution of starch accompanying a decrease in temperature is known to be independent of the formation of fat; in the potato, for example, the 'sweetening' depends, at temperatures just above 0° C., on the formation of sugar out of starch (MÜLLER-THURGAU, 1882), which is promoted by quite low temperatures. This disappearance of starch at low temperatures cannot be accounted for by special peculiarities of diastase.

We have now obtained a knowledge of the changes which certain organic compounds undergo in the green plant; at the same time, we have glanced at only a relatively limited number of chemical substances, namely, the proteid bodies and the crystalline nitrogenous organic substances resulting from their de-

composition, the fats and the carbohydrates (we will deal with the organic acids in succeeding lectures). It requires no depth of chemical knowledge to know that the wealth of chemical compounds in the plant is not thereby exhausted. One need only refer to the odours which are peculiar to so many plants to recognize at once a large series of bodies of wide distribution, such as ethereal oils, resins, &c. Further, we must not forget the colouring matters, which make their appearance in such variety, not only in the flower region, but in the vegetative organs as well, taking the place of chlorophyll. Finally, we are acquainted with bodies to which many plants owe their poisonous or curative powers, the glucosides and alkaloids. Since such substances appear to occur again and again under the same conditions in the same plant, they, as well as sugar, proteid, &c., must be products of metabolism, and the same questions must be asked about them, viz. how are they formed? What becomes of them? What significance have they in the plant economy? Although we have not entered into a discussion of these questions in our consideration of the metabolism of the green plant it is not because they are without interest, but because the researches which have been hitherto carried out on them have led to no, or only to indifferently, conclusive results. We know that many of these bodies do not undergo any further transformation in the plant, we may consider them as final products of no value, as excreta in short. [Many glucosides, hitherto regarded as waste products, act as reserves according to WEEVERS (1904.)] Such a purely chemical conception is, doubtless, one-sided. The cell-wall in the majority of cases is not further altered in the course of metabolism, but no one would consider it as an excretion, since it is of the very highest importance in the life of the organism. Many such examples might be brought forward; and it follows that the so-called biological significance of materials demands notice, and this is true especially for such substances as scents, colouring matters, alkaloids, and glucosides, and their significance has been often looked for and found with greater or less success. To enter into that aspect of our problem would take us too far and so we must content ourselves with this brief summary. We may at least draw attention to some of the more important works on the chemistry, physiology and biology of these metabolic end-products [CZAPEK, Biochemie], such as *ethereal oils*, *resins*, &c. :—TSCHIRCH, 1900: Die Harze und die Harzbehälter, Berlin; H. MÜLLER, 1873: Die Befruchtung der Blumen, Leipzig; DETTO, 1903: Flora, 92, 147. *Colouring matters* :—ROSCOE: Ausf. Lehrbuch d. Chemie, vol. 8, 1901; H. MÜLLER, 1873: Die Befruchtung der Blumen, Leipzig; STAHL, 1896: Bunte Laubblätter (Annales Buitenzorg, 13, 137); *Alkaloids and glucosides* :—ROSCOE: Ausf. Lehrbuch d. Chemie, vol. 8, 1901; VAN RIJN, 1900: Die Glykoside, Berlin; PICTET, 1900: Die Pflanzenalkaloide, Berlin; STAHL, 1888: Pflanzen und Schnecken (Jen. Ztschr. f. Naturw. 22).

Bibliography to Lecture XIV.

BALICKA-IWANOWSKA. 1903. Bull. Acad. Cracovie.
CZAPEK. 1897. Sitzungsber. Wien. Akad. Math.-nat. Cl. 106, I, 117.
FISCHER, ALFR. 1890. Jahrb. f. wiss. Bot. 22, 73.
FRUWIRTH and ZIELSTORFF. 1901. Versuchsstationen, 55, 9.
GAUCHER. 1900. Annal. Sc. nat. VII, 12, 241.
HABERLANDT. 1883. Sitzungsber. Wien. Akad. Math.-nat. Cl. I, 87, 1.
[HABERLANDT. 1904. Physiol. Pflanzenanatomie, 3rd ed. Leipzig.]
HARTIG, TH. 1858. Bot. Ztg. 16, 332.
[KNIEP. 1905. Flora, 94, 129.]
KÖRNICKE. 1901. Sitzungsber. Niederrhein. Gesell.
KOSUTANY. 1897. Versuchsstationen, 48, 13.
MIEHE. 1901. Flora, 88, 105.
MÜLLER-THURGAU. 1882. Landw. Jahrb. 11, 751.
[NATHANSOHN. 1904. Jahrb. f. wiss. Bot. 39, 607.]

[Niklewski. 1905. Beih. z. bot. Centrbl. 19, 1.]
Pfeffer. 1872. Jahrb. f. wiss. Bot. 8, 485.
Pfeffer. 1873. Monatsber. Berl. Akad.
Pfeffer. 1892. Stud. zur Energetik, Abh. Sächs. Gesell. 18, 275.
[Prianischnikow. 1904. Ber. d. bot. Gesell. 22, 35.]
Ramann, E. 1898. Zeitschr. f. Forst- u. Jagdwesen, Rev. Bot. Ztg. 56 231.
Schimper. 1885. Bot. Ztg. 43, 756.
Schulze, E. 1898. Zeitschr. f. physiol. Chem. 24, 18; ibid. 30, 241.
Strasburger. 1891. Bau u. Verrichtung d. Leitungsbahnen. Jena.
De Vries. 1885. Bot. Ztg. 43, 1.
[Wächter. 1905. Jahrb. f. wiss. Bot. 41, 165.]
[Weevers. 1904. Jahrb. f, wiss. Bot. 39, 229.]
Wehmer. 1892. Landw. Jahrbücher 21, 513.
Wortmann. 1890. Bot. Ztg. 48, 581.

LECTURE XV

THE ACQUISITION OF CARBON AND NITROGEN BY HETEROTROPHIC PLANTS

We shall now leave autotrophic plants and turn to the consideration of *heterotrophic* forms, that is to say, forms which have no power of forming carbohydrate from carbon-dioxide, nor the capacity for building up proteid out of nitrates or ammonia. They are dependent on previously manufactured organic substance, and also, in nature, on the nutriment they are able to take from other and autotrophic plants. In reality the contrast is not so sharply defined as it appears. In the first place, so far as the acquisition of carbon is concerned, only certain definite cells in autotrophic plants are really autotrophic, i. e. those which contain chlorophyll, all others are actually heterotrophic. We have seen that all subterranean organs, even the aerial stem itself, all growing regions and growing points, embryos, &c., are entirely dependent on already constructed organic substances. It may be further noted that the foliage leaf even, the specific organ for autotrophic nutrition, *may*, under certain conditions, be constructed exclusively out of carbohydrates, &c., brought to it from without (Jost, 1895). Although it is impossible in general to nourish higher plants in a purely heterotrophic manner in the absence of carbon-dioxide, the reason lies rather in the purely experimental difficulty of the research than in the natural and fundamental difficulties of the case. In some cases (Laurent, 1898) these difficulties may be overcome. [Laurent has shown in a recently published work (1904) that in the case of maize seedlings fed on sugar in the dark, the increase in weight always remains quite small.] Typical autotrophic plants in nature certainly live on carbohydrates manufactured by themselves, but there are also nontypical forms which, according to external conditions, are able to exist either in an autotrophic or heterotrophic manner (*Euglena*: Zumstein, 1899). [Artari (1899 and 1904) showed that the growth of certain lower Algae could be furthered by adding sugar.]

Since, then, the contrast between autotrophic and heterotrophic organisms is not so fundamental as it at first appears, we need not expect to find any entirely new feature in the nutrition and metabolism of heterotrophic plants. At the same time it is the proper course for us to devote a special section to the treatment of heterotrophic plants, since they exhibit in many respects peculiar conditions of life, and are much better adapted for the study of many problems in nutrition than are autotrophic plants.

Not infrequently the plant exhibits many diagnostic characters both in form and in mode of life which serve as criteria for determining whether it is

nourished in an autotrophic or a heterotrophic manner. Since the decomposition of carbon-dioxide is dependent on the presence of chlorophyll we may conclude from the absence of that colouring matter that the organism is of necessity obliged to fall back on organic materials containing carbon. Experimental observations on a large number of Bacteria and Fungi have fully confirmed this conclusion in the great majority of cases. On the other hand, the constant occurrence of an organism in soil which is rich in organic material suggests that it may live heterotrophically even though chlorophyll be present. Although the plant be dependent on nitrogen, sulphur, phosphorus, or other ash constituents in an organic form, it does not always follow that it also requires the carbon to be in an organic combination. The most remarkable condition is where another living organism, animal, or plant serves as a substratum rich in organic nutriment, where, in a word, the mode of life is *parasitic*. A large number of Fungi as well as certain higher plants, e. g. *Lathraea* and *Orobanche*, exhibit this type of heterotrophic life; in these, chlorophyll is for the most part wanting. A colourless parasite must, so far as nutrition is concerned, obviously behave just like the colourless member of an autotrophic plant, e. g. the root. It might, therefore, be thought that it would serve the purpose if we were to treat such parasites as an appendix to our study of autotrophic plants. In reality, however, we know very little indeed as to their nutrition; we are much better acquainted with the behaviour of certain Fungi and Bacteria which live on dead organic matter. Whilst parasites are always present on certain definite plants, often on a single species or variety, many *saprophytes*, that is to say, heterotrophic organisms which live on dead organic materials, appear on the most varied substrata, and thus are specially adapted for the study of the nutrients which make life possible for them. We shall begin with these plants and especially with their dependence on carbon in the organic form. [BENECKE has recently published (1904) an important and comprehensive memoir on the nutrition of Fungi.]

The requirements of Mould-fungi as regards mineral matters have already been referred to; it will be sufficient, therefore, to note here that essentially the same substances are needed by them as by higher plants. The only difference is that the Fungi require only *one* of the alkaline earths, calcium or magnesium, whilst higher plants require *both*. In order to study the sources of carbon employed by our ordinary moulds we may select a nutritive solution which, in addition to minerals, contains nitrate of ammonia to meet the demand for nitrogen, adding to the solution different bodies containing carbon, and introduce a few spores of *Aspergillus niger* or of *Penicillium glaucum*. According to the way in which the fungus grows we may readily draw conclusions as to the nutritive value of the source of carbon supplied. We are able to determine, for example, that sugar is an excellent nutrient, but that many acids, such as formic or oxalic, are very inferior nutrients or have no nutritive value at all. Exhaustive studies on this question have been carried out by PASTEUR (1860 and 1862), NÄGELI (1879 and 1882), and REINKE (1883). These investigators have shown that an extraordinarily large number of carbon compounds may serve as nutrients to Fungi, e. g. carbohydrates, alcohols, organic acids, fats, amido-compounds, peptones, &c. We give below a summary by way of showing how varied in character these compounds are; the materials are arranged in descending order of their value as nutrients :—

NÄGELI (1882) gives the following series à propos of Fungi :—1. sugar; 2. mannite, glycerine, leucin; 3. tartaric acid, citric acid, succinic acid, asparagin; 4. acetic acid, ethyl-alcohol, quinic acid; 5. benzoic acid, salicylic acid, propylamin; 6. methylamin, phenol.

PFEFFER (Phys. I, 372), as a result of later experiments, rearranged the series as follows:—1. sugar; 2. peptone; 3. quinic acid; 4. tartaric acid; 5. citric acid; 6. asparagin; 7. acetic acid; 8. lactic acid; 9. ethyl-alcohol; 10. benzoic acid; 11. propylamin; 12. methylamin; 13. phenol; 14. formic acid.

Duclaux (1885, 1889) finds the following substances of special value to *Aspergillus*:—1. dextrose; 2. cane sugar; 3. lactose; 4. mannite; 5. alcohol; 6. acetic acid; 7. tartaric acid; 8. butyric acid.

Laborde (1897) has compared *Aspergillus* with another fungus (*Eurotiopsis gayoni*) and found that the latter was unable to make use of cane sugar and tartaric acid, although it accepted lactic acid which was quite unsuitable for *Aspergillus*. Finally Went (1901) obtained the following series for *Monilia sitophila*:—carbohydrates, acetic acid, mannite, glycerine, lactic acid, malic acid, ethyl-alcohol, ethyl acetate, tartaric acid; a number of other acids are poor nutrients, while formic and benzoic acids are of no use at all. [Certain Bacteria are able to exist with the aid of the traces of volatile organic compounds occurring in the air (Beijerinck, 1903). According to certain older experiments of Elfving this is true also of many Fungi.]

There is nothing to be gained by the citation of additional examples, since a comparison of the results obtained by different authors is at present unfortunately not possible, seeing that certain factors, of which we shall speak by and by, have not been studied with sufficient completeness. Thus, e. g. the *nutritive value* of a certain carbonaceous substance may depend on the age of the fungus, for it is not infrequently the case that during germination more exacting demands are made on such substances than later on; *Aspergillus*, e. g., *germinates* very badly in the presence of lactose and mannite, whilst a somewhat older plant thrives quite well in the presence of these bodies. In the second place, the *chemical reaction* of the substratum has to be noted. In this relation there is a noticeable difference between Fungi and Bacteria; the former prefer weak acid solutions, the latter weak alkaline. In both cases, however, an excess of free acid as well as of free alkali inhibits development. The *quality* of the *nitrogenous material* also has an influence on the nutritive value of any particular carbon compound. Thus glucose is the best source of carbon for *Monilia sitophila* when peptone is used as the source of the supply of nitrogen; but if aspartic acid be used instead of peptone, cane sugar is found to be far more valuable than glucose (Went, 1901). As may be easily understood the *concentration* of the nutritive solution is of importance, but Fungi have a wonderful capacity for adapting themselves to high degrees of concentration, as elsewhere occurs only in the case of germinating pollen-grains (Correns, 1889. [Molisch, 1893.]) The high osmotic activities of concentrated sugar solutions were alluded to earlier. Fungi germinating in such solutions must develop a much higher osmotic pressure than usually occurs in plant-cells, otherwise plasmolysis would be induced. Eschenhagen (1889), who carried out experiments on this subject in the Leipzig Institute, obtained the following values for the maximum degree of concentration (in weight per cent.) which ordinary Fungi could tolerate:—

	Glucose.	Glycerine.
Aspergillus niger	53	43
Penicillium glaucum	55	43
Botrytis cinerea	51	37

From these numbers we may calculate how great the pressure in the interior of the cells must be, since the osmotic value of the cell-sap in a turgid cell must always exceed that of the external solution.

[Raciborski (1905) has obtained even greater values, for he has shown that *Aspergillus glaucus* and a species of *Torula* could grow in concentrated salt solution; *Torula* germinated in a saturated solution of lithium chloride, that is to say, in a fluid which gives the highest osmotic pressure of all neutral salts.]

The adaptation to high degrees of concentration is apparently in many cases effected by the *formation* of unknown osmotically active bodies in the cell

(HEINSIUS, 1901), in other cases, by the *entry* of the nutritive solution ; and in Bacteria and Cyanophyceae especially, the protoplasm appears to be extraordinarily permeable. In addition to its osmotic effect the nutritive solution may have as well a poisonous action when certain variable concentrations are reached. A 10 per cent. solution of alcohol is in general injurious to Fungi, while a 2–4 per cent. solution is usually nutritive ; the maximum for butyric acid lies much lower, i. e. about 0·4 per cent. In such cases it is conceivable that the different organisms behave in different ways, and a certain form might be able to tolerate a *gradually increasing* concentration, which would be fatal if suddenly applied (compare MEISSNER, 1902).

Lastly, let us glance at the influence of temperature. As THIELE (1896) has shown, the temperature maxima suitable for the growth of *Penicillium* lie at variable heights according to the food materials provided ; development ceases about 31° in the presence of grape sugar, with formic acid at about 35°, and with glycerine at about 36°. Formic acid has, therefore, a greater nutritive value at high temperatures than glucose, whilst at ordinary temperatures it is nearly the worst, the glucose proving itself the best source of carbon.

All the points above indicated, and many others as well, must be taken into account in any renewed investigations if exact results are to be obtained as to the comparative nutritive value of the different compounds of carbon. Though definite results have by no means been obtained in all cases, still, from the researches already carried out, it may be concluded that a large number of compounds may act as nutrients to Fungi, but that their value is very unequal. The nutritive value of these compounds depends obviously on the nature of the compound itself, but the special peculiarities of the organism which it nourishes are also of importance. This latter condition becomes especially apparent when we compare ordinary Fungi, which we may term *omnivors*, on account of their ability to nourish themselves with the most varied food materials, with *specialists*, that is to say, such forms as are compelled, during their life, to use certain definite substances as food. Thus *Mycoderma aceti* thrives well on alcohol and acetic acid, substances which are of little value to other Fungi. *Bacillus perlibratus*, according to BEIJERINCK (1893), grows exceedingly well in acetic and malic acids, but seems to be unable to assimilate tartaric acid, whilst this latter substance is, as a rule, a better foodstuff and is especially acceptable to *Bacillus cyanogenus*. Many similar examples might be adduced, and we shall meet with other cases of 'specialism' in the course of our studies.

At the same time it must be remembered that there are many organic substances which are useless even to the most thoroughly omnivorous types. NÄGELI carried out experiments (1879) with the view of finding what part was played by the *constitution of the compounds* in determining whether they could be assimilated or not. It appeared that carbon could be assimilated whether it occurred in the combination CH_2 or CH ; in the combination CHOH it proved injurious, and when present as CO or CN it was found to be quite useless (NÄGELI, 1879, 401). There are, however, many exceptions known to this rule (REINKE, 1883, DIAKONOW, 1887, BEIJERINCK, 1901) ; thus, for example, urea $CO<\begin{matrix}NH_2\\NH_2\end{matrix}$ and oxalic acid $\begin{matrix}C-OOH\\|\\C-OOH\end{matrix}$ are capable of nourishing certain organisms, and we may yet, by careful research, be able to show that carbon may be assimilated when in combination with nitrogen. At present we are forced to the conclusion that the constitution of the compounds is not nearly so important as NÄGELI supposed. That this is so is shown by the fact that Fungi are able to obtain all their organic food just as well from methane derivatives (glucose) as from benzol derivatives (quinic acid). On the other hand, as the results of careful observation, it has been shown that Fungi possess an extraordinary capacity for distinguishing sub-

stances which our ordinary chemical reagents fail to differentiate; that of two bodies which have an entirely similar constitution and differ only in the spacial arrangement of their atoms, the one is assimilated easily, the other with difficulty or not at all. A well-known and typical example of the behaviour of such 'stereoisomeric' bodies has been given by PASTEUR (1858, 1860). He cultivated *Penicillium* in optically inactive racemic acid and showed that it was resolved into dextro- and laevo-tartaric acids, and that the dextrotartaric acid was used up first. Numerous similar examples have been discovered since then (PFEFFER, 1895), and it has been shown that many, though not all, organisms prefer definite optically active substances. There is, for example, a bacterium which behaves in exactly the reverse way to *Penicillium*, and which prefers the laevotartaric acid (PFEFFER, 1895), while *Bacillus subtilis* appears to have no preference for either. Similarly BUCHNER (1892) has observed that fumaric acid forms a good nutrient for *Aspergillus* and *Penicillium*, while the stereoisomeric maleic acid is known to be rapidly poisonous. Reference should also be made here to lactic acid and many of the glucoses, which we shall take a later opportunity of discussing.

Interesting as these conclusions are we do not obtain by their study any deeper insight into the reasons for the unequal metabolic value of nearly related bodies nor for the similar treatment of very different bodies; and yet elucidation of these facts must be obtained if we are to reach a clear understanding as to the mode of assimilation of food materials. Meanwhile our experiments with stereoisomeric bodies offer us valuable suggestions in other respects. They show, for example, how well the power of selection is developed in Fungi. *Aspergillus* is able to distinguish not only between dextro- and laevo-tartaric acids but also between entirely distinct substances. Out of a nutritive solution containing much glucose and some glycerine, it selects first of all the more valuable food-material, viz. the glucose. It may be said indeed that, in the presence of glucose, glycerine is not employed at all. The converse, however, does not hold good; the slightest traces of dextrose are greedily absorbed, although glycerine be present in quantity. Similarly, PFEFFER (1895) has shown that glycerine is excluded from metabolism in the presence of peptone and lactic acid in the presence of dextrose.

Let us glance now at the requirements of heterotrophic organisms for *nitrogen*. In the nutritive solutions we have employed hitherto we have in general presented the nitrogen in the form of nitrate of ammonia, and we saw that the requirements for nitrogen were met in this way, and that proteid was undoubtedly manufactured. We have now to ask whether this is the only and the best form in which one may offer nitrogen to Fungi. One very important question is whether nitrate will act without ammonia, and whether Fungi, like autotrophic plants, prefer a nitrate to an ammonium salt. As a matter of fact, research has shown that different Fungi and Bacteria behave in totally different ways in relation to nitrogen, so that they have to be arranged in several groups (compare BEIJERINCK, 1890, FISCHER, 1903, p. 96; [BENECKE, 1904]).

1. *Nitrate Organisms.* These thrive in the presence of nitrates quite as well as, if not better than, along with other compounds. To this group belong the Fungi *Alternaria tenuis*, *Mucor racemosus*, *Aspergillus glaucus* (LAURENT, 1889); and among Bacteria: foecal Bacteria (JENSEN, 1898), *Bacillus pyocyanus*, and *Bacillus fluorescens*. Some employ *nitrites*, e. g. *Bacillus perlibratus* (BEIJERINCK, 1893) and a fungus described by WINOGRADSKY (1899).

2. *Ammonia Organisms.* These develop in the presence of nitrates but thrive much better with ammonia. To this series belong, e. g. *Eurotiopsis*, *Aspergillus niger*, yeast and *Bacillus subtilis*.

3. *Amide Organisms. Bacillus perlibratus*, *Bacillus typhi* and *Rhizopus oryzae* grow better with asparagin than with ammonia. Other acid amides and amino-acids appear to operate in a similar manner.

4. *Peptone Organisms.* Scarcely any growth takes place with asparagin or ammonia ; nor can proteid replace peptone. Examples : *Bacillus anthracis*, *Bacillus proteus*, lactic acid Bacteria (BEIJERINCK, 1901).

5. *Proteid Organisms. Micrococcus gonorrhoeae* and *Bacillus diptheriae* require proteid and are unable to live in peptone or other nitrogenous substrata. They certainly exist in nature as parasites only and, strictly speaking, do not belong to this category.

6. Mention must also be made of organisms which use the free nitrogen of the air in preference to other nitrogenous material. We shall refer to them elsewhere.

The types which have been indicated show that among Fungi and Bacteria there are all sorts of transitional forms, from those which are able to assimilate nitrogen just like autotrophic green plants to such as are dependent on previously elaborated proteid. We may if we choose, therefore, designate the nitrate and ammonia organisms as autotrophic so far as their relation to nitrogen is concerned, and term the others heterotrophic. But just as we found that the requirements of the plant, *re* carbon, often depended on the combination in which nitrogen was presented, so conversely the nitrogen requirements depend on the source of carbon. Thus A. FISCHER (1897, p. 53) found that *Bacillus coli*, *Bacillus subtilis*, and *Bacillus pyocyanus* could use *nitrate* in presence of glucose ; but if glycerine were substituted for glucose, *Bacillus pyocyanus* alone thrived ; the others used ammonia exclusively as a source of nitrogen when glycerine formed the source of carbon. When proteid or peptone was supplied as nutritive nitrogen, an additional supply of a special source of carbon was found to be unnecessary ; frequently the same was found to be true of asparagin. A comparative research on the nitrogen requirements of different Fungi would be of special value since the results hitherto obtained are exceedingly fragmentary, and similar researches are needed on the nitrogen requirements both of omnivors and of specialists.

Nor are we able to answer comprehensively the problem as to the best combinations of carbon and nitrogen, although it is generally held that a nutritive solution containing peptone and glucose acts best. On the other hand, CZAPEK (1902) has shown in the case of *Aspergillus* that the amino-acids are preferable to peptone in presence of glucose. Taking into account the statements in the literature, however, it cannot be said that any law of general applicability has as yet been formulated. BEIJERINCK (1891) found, for example, that peptone alone (as a source of carbon and nitrogen) was a better medium for *Bacillus cyaneofuscus* than asparagin and glucose, and WENT (1901) demonstrated in the case of *Monilia* that when glucose formed the source of carbon, peptone was preferable to all other substances ; with asparagin it reached only a third of the increase in weight gained when peptone was supplied, while leucin was found to be far inferior to potassium nitrate. Ammonium salts of acetic acid, tartaric acid, &c., were found to be specially unsuitable combinations of carbon and nitrogen, although many Fungi for long maintained their growth in them.

The ubiquity of Fungi and of biologically related plants is due to their capacity for living on the most varied organic materials, together with their remarkable power of adapting themselves to highly concentrated nutritive solutions. The general occurrence of dead vegetable tissues renders the existence of Fungi possible in most situations, and so we see that on dead leaves, twigs, and fruits, a covering of Fungi soon appears, provided only the general conditions be sufficiently moist. Dead animals also, as well as animal excrement, form a suitable medium for the growth of Fungi, so long as the reaction be acid, as is generally the case in plant débris ; when the reaction is alkaline, on the other hand, Bacteria are the dominant organisms. Under the influence of these microscopic forms a decomposition of the débris of the higher organ-

isms takes place (which we will consider in greater detail later on) which frequently leads to the formation of humus. Humus again forms the home of a large number of Fungi and also of Phanerogams, which, like *Monotropa* and *Neottia*, are heterotrophic in their mode of nutrition owing to their possessing no chlorophyll. Leaving on one side for the moment the Phanerogams, whose complicated nutritive relations we shall consider later, there are numberless Basidiomycetes at least, of which it may be said that they obtain all the organic materials they require from humus, although what precisely these substances consist in we are quite ignorant. The peculiar humus substances which are soluble in alkalis cannot, according to REINITZER (1900), at least in most Fungi, act as a source of carbon, although they may serve as a source of nitrogen (compare NIKITINSKI, 1902); REINITZER, who experimented with *Penicillium*, holds that possibly certain 'specialists' may obtain their supply of carbon from humins.

We do not know precisely what other organic substances occur in humus besides humins, and although we are unable to extract any useful nutritive materials from it by means of ordinary chemical media, it is quite likely that many of the Fungi are able to do so by excreting enzymes with dissolving capacities. These enzymes are among those already studied, viz. diastases and sugar-splitting enzymes, also cytase and protease. (Many other enzymes occur in *Monilia*; compare WENT, 1901). On the enzymes occurring in Fungi a number of interesting observations have been made, two of which only we shall refer to here. In the higher plants we found cytases occurring as a rule only when required to dissolve *reserve* cellulose; the cellulose of ordinary cell-walls in the presence of these enzymes remains as originally formed completely untouched; it is not dissolved and reabsorbed before the fall of the leaf, and thus when the leaves and branches fall a large amount of organic material is lost. In many Fungi, however, not merely those which live as 'specialists' on wood, such as *Merulius lachrymans*, the dreaded 'dry rot', and other wood destroyers, but also in Fungi in general, the power of dissolving cell-walls has been definitely proved to exist. This capacity is obviously developed in many cases only to enable the fungus to enter the interior of the cell; the dissolution of the cell-wall is subsidiary to the chief object, viz. to reach the cell contents, starch, &c. In other cases the fungus apparently lives chiefly on the cellulose, and is even able (CZAPEK, 1899) to make use of lignified walls, effecting, by means of a special enzyme, a splitting off of the cellulose from the etherial substance combined with it (hadromal, compare p. 70). The cellulose is assimilated, but the hadromal remains unused. In this capacity possessed by these Fungi we have *one* mode of destroying cellulose in nature; in Lecture XVII we shall have to speak of another method of achieving the same result. Were it not for such decompositions the surface of the earth would soon be entirely covered with thick layers of cellulose.

Another observation which may be referred to deals with the *excretion* of diastase. So far as seedlings are concerned a controversy still exists as to whether diastase may be excreted from living cells, since it is often assumed that it cannot pass through the cell-wall (compare p. 156, Lecture XII). It cannot be doubted that in the case of Fungi and Bacteria such an exudation of diastase through the cell-wall does take place. WORTMANN (1882), and especially PFEFFER (1896) and KATZ (1898), have shown that the production of diastase is not a constant characteristic of certain Fungi, but that it can be induced or inhibited by external conditions. In the abundant presence of various sugars but not of all suitable nutritive sources of carbon, no diastase is formed; in *Penicillium*, for example, a 2 per cent. solution of sugar is sufficient to inhibit its formation. *Bacterium megatherium* behaves in a similar manner, while in the case of *Aspergillus* a 30 per cent. solution of sugar only retards, but does not inhibit, the formation of diastase. The

formation of the enzyme is often, though by no means always (WENT, 1901), *regulated* by the *need* for it; when the product of the activity of the enzyme, or perhaps only a body like it, is present in the plant, the enzyme is not required. Doubtless the formation and dissolution of starch, for example, is regulated in higher plants in this way, and similar self-regulating processes are likely to occur, other than those associated with metabolism.

Without entering further into a discussion of the other enzymes (on which an extensive modern literature exists, e.g. FERMI and BUSCAGLIONI, 1899, SANGUINETTI, 1897, MALFITANO, 1900, KOHNSTAMM, 1901, BRUNSTEIN, 1901), we can easily see how greatly the possession of such a secretion capable of bringing about extra-cellular solution facilitates the distribution of Fungi in nature, where suitable nutritive solutions are not always to be obtained ready to hand. They possess not only *dissolving* but also splitting enzymes as well. Disaccharides must be split up before they can be used, for if an organism such as *Bacillus perlibratus* (BEIJERINCK, 1893) possessed no sugar-splitting enzymes it could thrive only on dextrose and levulose, but would be debarred from using maltose, cane sugar and lactose.

The heterotrophic plants we have hitherto been considering are saprophytes, i. e. they exist in nature on the dead remains or the excreted metabolic products of the animal and plant worlds. In a certain sense we may describe the biological group of plants known as *carnivors* as an intermediate class between the saprophytes which we have been studying and the parasites yet to be discussed.

These much investigated and well-known *carnivorous*, or, as one may more specifically term them, *insectivorous plants* have excited the greatest interest not merely among botanists but the general public as well, on account of the contrivances by which they obtain possession of their nutriment and by the methods they use in digesting it. But from a purely physiological point of view they are so gradually transitional to other types that they might be classed equally correctly under saprophytes or under autotrophic plants. We cannot enter here into a description of the morphological characters of these plants; we may refer to Figs. 33–35 and to the special treatises on the subject, especially those of GOEBEL (1891–3), and only remark that in order to catch small insects essentially three types of apparatus are employed, viz. (1) pitchers, or trap-like cavities, such as the pitchers of *Nepenthes* (Fig. 33), *Sarracenia* and *Cephalotus*, or the bladders of *Utricularia* (Fig. 35); (2) closing traps, that is, organs which catch insects by active movements, *Dionaea* (Fig. 34) and *Aldrovanda*; (3) sticky hairs (*Drosera*, Fig. 156, Lecture XXXVIII), *Drosophyllum*, *Pinguicula*. Combinations of these contrivances also occur.

In certain simple cases, such as the bladders of *Utricularia*, the animals remain for a long time alive, die at last of hunger and decompose *in situ* as the result of the action of Bacteria. We may assume that their excrement at first and, later, the products of the decomposition of their bodies are used by the plant as sources of nitrogen, so that a truly carnivorous habit does not here exist. A similar state of affairs appears to hold good for *Sarracenia* and *Cephalotus*, and hence we may group these plants in the same category with others which possess water reservoirs, especially the Bromeliaceae already mentioned and *Dischidia rafflesiana*, with its remarkable water pitchers; in all these cases animals are invariably present in the water reservoirs, their products of decomposition being in course of time absorbed by the plant. The typical carnivorous plants are distinguished, however, by the fact that they secrete *proteolytic enzymes*, for the most part along with acids, by means of which they are able to dissolve *proteid* materials. Further differences exist between the different types, inasmuch as in some species the protease and the acid are *always* being secreted, whilst in other cases one or both substances are produced only in response to a *stimulus*, especially

the chemical stimulus resulting from the presence of a digestible substance. As yet, however, we know very little either about the enzyme or the acid. According to one account, formic acid is declared to be present, but more recently this has been denied. VINES's (1897–1902) discovery that *Nepenthes* excreted a *tryptic* ferment has been lately questioned by CLAUTRIAU (1900), who thought he had proved the presence of a *pepsin*. It is true that minute particles of meat,

Fig. 33. Pitcher of *Nepenthes*, partly cut open to show contents. The basal region is occupied by a fluid excreted from special glandular hairs in which the animals which fall into the pitcher are digested. From the Bonn Textbook.)

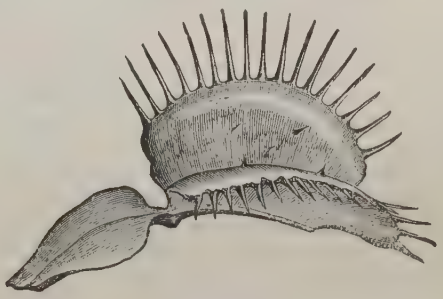


Fig. 34. Leaf of *Dionaea muscipula*. When the hairs on the upper side of the leaf are touched the two half blades instantly come together. (From the Bonn Textbook.)

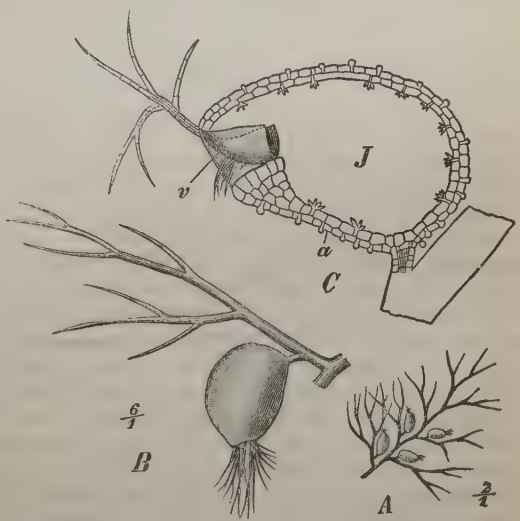


Fig. 35. *Utricularia vulgaris*. *A*, portion of the leaf with bladders; *B*, portion of a leaf with bladder; *C*, bladder in longitudinal section (× 28); *v*, trapdoor; *a*, wall; *f*, central cavity. (From the Bonn Textbook.)

fibrin, &c., are dissolved rapidly by *Nepenthes* and by *Drosera*, and that, too, certainly without the help of Bacteria. Many of the secretions of carnivorous plants have, in addition, antiseptic characters, so that micro-organisms are quite unable to exist in them.

The products resulting from the dissolution of proteid are absorbed either by the secretory hairs which produce the enzyme or by other special trichomic structures. Digestion and absorption take place often very rapidly. Thus DARWIN (1876) observed that small cubes of white of egg were dissolved in the course of one or two days, and that the gelatinous fluid resulting was completely absorbed in about three days.

The question whether carnivorous plants are benefited by being fed on insects has often been discussed. It is a well-established fact that they can not only exist but also thrive without any such nutriment. On the other hand, it has often been shown that such food materials produce a favourable result when given in sufficient amount. In the case of BÜSGEN'S (1888) researches, for example, the increase in growth of fed *Utricularia* shoots was double that of the unfed; and the same author (1883) showed that *Drosera* exhibited remarkable results after being nourished on meat from the seedling stage right up to the formation of seed. The dry weight of the plants which had been fed was one and a half to three times that of the unfed; the fed plants had three times as many inflorescences, and formed five times as many capsules. There can be no doubt that carnivorous plants are autotrophic so far as the acquisition of carbon is concerned; all of them have abundant chlorophyll and can thrive without receiving any proteid food. The favourable action of the latter cannot well depend on the gain in organically combined carbon; it is more probable that it originates from the nitrogen supplied or certain nutritive salts. It is possible that feeding with insects is beneficial simply because a *larger amount* of combined nitrogen and materials of the ash is introduced in this way into the plant than could be obtained from the *soil only*. It is even more probable, however, that the *quality* of the materials which are absorbed by the leaves is of significance; for instance, the obtaining of organically combined phosphorus or organic nitrogen may be the end specially aimed at. In the latter case, which, without more exact proof may be regarded as the point of chief importance, we must regard peptones as especially valuable. It is, in fact, perfectly obvious that carnivorous plants are peptone-feeders, or in other words that they are better nourished when the nitrogen is in the form of peptone than in that of nitrate or ammonia. This idea is well worthy of investigation; meanwhile, we must depend on analogies and it is important to remember that there are peptone organisms not only among Fungi, which are also dependent on carbon in the organic form, but also among green autotrophic plants. BEIJERINCK (1890) and ARTARI (1899) have shown that certain Lichen-Algae (compare Lecture XIX) prefer peptone to any other compound of nitrogen.

The biological position of carnivorous plants is at present somewhat indefinite, still it is fairly well settled that their proper place is among what may be termed 'nitrogenously heterotrophic' plants. As already remarked, we have another series of heterotrophic plants to consider, namely *parasites*. These forms are united with saprophytes by many transitions. Fungi are known (compare DE BARY, 1884) which live as a rule saprophytically, but which still have the power of penetrating living organisms and of taking their nourishment from them. As examples of such 'facultative parasites' may be taken *Penicillium glaucum* and other Mould-fungi which enter into wounds in ripe fruit and cause putrefaction in them; further, *Sclerotinia sclerotiorum* may be mentioned, as a fungus which can carry out its entire cycle of development as a saprophyte, and does so in nature not infrequently, but which, after attaining sufficient vigour, can live also as a parasite on many plants. The converse condition is also known, viz. Fungi, which usually live as parasites, but which can nourish themselves saprophytically ('facultative saprophytes,' e. g. *Phytophthora omnivora* and many Bacteria), and research has certainly shown that very many, if not all, parasites can grow and increase without the aid of their usual hosts. The probability of such a result is very varied in different degrees of parasitism.

The lower stages of parasitism include such Fungi as appear to attack many different species of plants, as, for example, the already mentioned *Phytophthora omnivora*, which is parasitic on *Fagus*, *Sempervivum*, *Oenothera* and other plants, and *Sclerotinia sclerotiorum*, which is apparently able to attack all succulent parts of plants. These parasites are distinguished from ordinary

saprophytes by their power of entering into the host plants, killing their cells, and robbing them of their nutritive contents. If these *omnivorous* parasites avoid individual plant species one must consider it as due to their want of ability to enter them and not that they cannot find in them food materials suited to their wants. It has indeed been proved that facultative saprophytes make no demands on any *definite* nutritive material. It is otherwise with Fungi which are limited to a single family, genus, or species, which are particular in the selection of a host, often indeed confining themselves to a single species ; these types represent a higher grade of parasitism. As examples we may cite *Cordyceps militaris*, a parasite on certain insects ; many Uredineae and Ustilagineae on specific representatives of some plant families ; *Cystopus portulacae*, which occurs only on *Portulaca* ; *Uromyces tuberculatus*, only on *Euphorbia exigua* ; *Laboulbenia baeri* only on the common house-fly. Undoubtedly this limitation to one or a few organisms which act as hosts depends on the fact that these hosts are not only specially suited for the entrance of the parasites, but chiefly that they provide some peculiar and *special* nutrient material required by the fungus, although we cannot say exactly what its nature is, or at best can only guess.

The number of parasites among Fungi is very great. The Fungi are, indeed, generally speaking, so far as they do not exhibit special features of which we shall speak later, typically heterotrophic plants. There are fewer heterotrophic plants, and more specially parasites, among Phanerogams, but the latter exhibit so many variations that they claim special mention. First of all, we have *Lathraea* and *Orobanche*, which, owing to their want of chlorophyll, remind one of Fungi, and in many tropical forms, e. g. the Rafflesiaceae, the likeness to Fungi appears even in the structure of the vegetative organs. The use of carbon-dioxide by these plants is naturally quite impossible ; in relation to the acquisition of carbon, nitrogen and minerals, they are completely dependent on their host plants, and generally speaking, they possess no organs with which to absorb materials from the soil. How much they are dependent on their host plants is especially well seen in their germination, which, in the case of *Lathraea* and *Orobanche*, takes place only when the seed is placed in immediate proximity to the root of the host-plant ; in such cases there must be some definite substance given off by the root which permits of germination taking place (Lecture XXV). In the same group of parasitic Phanerogams occurs also *Cuscuta*. This plant cannot live without a host, although isolated shoots may become green (Peirce, 1894). It may be assumed that the chlorophyll arising in this way is functional, but that the products of assimilation are insufficient in quantity to maintain the plant in life. We must look upon the capacity for forming chlorophyll as an indication that *Cuscuta* has been evolved from chlorophyll-bearing plants ; like *Lathraea*, it in all probability quite lost the power of forming chlorophyll at a very early period in its phylogenetic history. That is certainly not essential since a whole series of Phanerogams have not lost it in their gradual transition to a parasitic mode of life, as, for example, many Scrophulariaceae (*Euphrasia, Rhinanthus, Bartsia, Tozzia*), Santalaceae (*Thesium*) and Loranthaceae (*Viscum, Loranthus*). Among these the Rhinantheae have been most closely studied. With the exception of *Tozzia*, they are in their germinating stage independent of the presence of a host plant and can often carry on their development for a certain time without any host at all. *Tozzia* has progressed farthest in parasitism and is most dependent on outside help ; the other extreme is occupied by individual species of the genus *Euphrasia* (*E. odontites, E. minima*), which can reach the flowering and fruiting stage without a host, while others, e. g. *E. rostkowiana*, although able to germinate without the aid of a host, develop only into dwarf forms. These green parasites are not very particular as to what host they select ; one reason for this is that their seeds, if sown sufficiently closely, hold

on to others of the same species, as L. Koch (1888) was the first to show, and that *one* of several seeds bound together by haustoria develops at the expense of the rest.

Heinricher, to whom we owe exhaustive researches on the Rhinantheae (1897,onwards) has endeavoured to prove that these parasites decompose carbon-dioxide by means of chlorophyll in the normal way. This cannot be proved with certainty, and researches are especially wanting as to whether the products of assimilation so formed are *quantitatively* sufficient for their needs (compare what has been said above as to *Cuscuta*). It is possible that the chlorophyll function may be present but in a weakened form, that it is insufficient for the plant's requirements, and that the plant must have recourse to ready formed carbohydrate. On the other hand, one may explain the parasitism of these plants by saying that they are dependent on their hosts only for nitrogenous material, perhaps in the form of proteid or amide bodies, or for the materials of the ash. Heinricher, on account of the quantity of nitrates in parasites, decides in favour of the latter possibility and concludes that these green parasites employ their hosts as a source of unelaborated sap only. The question is a purely experimental one and the decision will depend on the results of such experiments.

The common mistletoe is still imperfectly known so far as the physiology of its nutrition is concerned. Owing to the fact that the union between host and parasite is limited to the water-carrying vessels, we are led to believe that *Viscum* takes only water and inorganic salts from its host ; and such a relation is more probable in a plant that lives high up on trees than in forms like *Euphrasia*, which has roots in the soil and is partly, at least, provided with roothairs. *Viscum* may be conceived as a plant, originally epiphytic, which has surmounted the difficulty of the deficiency of water and salts—against which most epiphytes have to contend—by attaching itself to the vascular systems of other plants. This conception has not, however, been substantiated, and here also experimental research is necessary.

Exhaustive morphological and developmental studies on these interesting phanerogamic parasites are to be found in the following works :—

Orobanche : Koch (1887) ; *Lathraea* : Heinricher (1895) ; *Cuscuta* : Koch (1880), Peirce (1894).

Rhinanthaceae : Koch (1889 and 1891), Heinricher (1897, 1898, 1901) ; *Loranthaceae* : Pitra (1861).

The difference between autotrophic and heterotrophic plants, it must once more be clearly pointed out, lies solely in the *mode of absorption* of nourishment, and consequently we may speak of *unicellular green organisms* only as autotrophic, for there the *whole* plant is concerned ; on the other hand, in the case of the higher plants only certain parts are autotrophic, the leaves especially, whilst others, as, for example, the roots, are completely heterotrophic. So far as regards the further alteration of the organic compounds of carbon and nitrogen, it is quite immaterial whether these are used for constructive purposes in the regions where they are made or whether they be translocated in an already prepared condition. There is no essential difference between the *metabolism* of heterotrophic and of autotrophic forms.

As in green plants, so in Fungi, the nutritive substances are employed in the construction of the body, are stored in reserves or, when of no more use, are transformed into waste products ; that is to say, we have here also to distinguish (1) plasta ; (2) reserves ; (3) translocatory materials ; (4) excreta. On the whole the cell of the fungus is constructed out of the same kind of materials as that of the Phanerogam, and although there are deviations in individual cases, e. g. in the occurrence of chitin in their cell-walls, still we need not go further into the matter, since, both in these as in the higher plants, we are unacquainted with the conditions of origin of the different constituents of the cell. The

principal agreement with autotrophic plants lies, in the first instance, in the nature of the reserve substances. In addition to *nitrogenous* we also find *non-nitrogenous* reserves in Fungi, and among these the fats are especially widely distributed; on the other hand, since chromatophores are entirely absent, Fungi form no starch. In regions where temporary or more permanent storage of carbohydrates might be expected to take place we frequently find starch replaced by *glycogen*, a substance which occurs in animals also. In yeast-cells glycogen (LAURENT, 1890, MEISSNER, 1900) is formed often in considerable quantity from the sugar present in the nutritive solution, and also apparently from various organic acids. Its accumulation in organs which are capable of growing in length with great activity in a short time, e.g. the stipe of *Phallus* (CLAUTRIAU, 1895), is especially remarkable; during this growth glycogen is altered just in the same way as starch would be under similar conditions in the stems of Phanerogams, in order to provide material for the formation of cell-walls.

Glycogen in its composition is closely related to starch, but it is soluble in water. Its large molecule makes it incapable of diffusing through either protoplasm or cell-wall, and it is for that reason well adapted to act as a reserve substance. It cannot wander from cell to cell; it must first of all be transformed into sugar by means of some enzyme related to diastase. It cannot be employed directly as a nutrient by yeast, since the enzyme cannot be excreted from the cell.

It is unnecessary for us to discuss the other metabolic processes in the fungus cell since, as we have said, they agree entirely with the corresponding processes in autotrophic Phanerogams.

Bibliography to Lecture XV.

ARTARI. 1899. Bull. naturalistes de Moscou, No. 1.
[ARTARI. 1904. Jahrb. f. wiss. Bot. 40, 593.]
DE BARY. 1884. Vgl. Morph. u. Biol. d. Pilze. Leipzig.
BEIJERINCK. 1890. Bot. Ztg. 48, 766.
BEIJERINCK. 1891. Ibid. 49, 705.
BEIJERINCK. 1893. Centrbl. Bakt. 14, 834.
BEIJERINCK. 1901. Archives néerland. II, 6, 212.
[BEIJERINCK and DELDEN. 1903. Centrbl. Bakt. II, Abt. 10, 33.]
[BENECKE. 1904. Lafar's Handb. d. techn. Mykologie, 1, 303–429.]
BRUNSTEIN. 1901. Bot. Centrbl. (Beihefte) 10, 1.
BUCHNER. 1892. Ber. d. chem. Gesell. 1161 (quoted by Pfeffer, 1895).
BÜSGEN. 1883. Bot. Ztg. 41, 569.
BÜSGEN. 1888. Ber. d. bot. Gesell. 6, LV.
CLAUTRIAU. 1895. Acad. de Belgique, Cl. d. sc. (Koch's Jahresbericht, 6, 51).
CLAUTRIAU. 1900. Mém. couronn., in 8°, Acad. Belgique, 59.
CORRENS. 1889. Ber. d. bot. Gesell. 7. 265.
CZAPEK. 1899. Zeitschr. f. physiol. Chem. 27, 141.
CZAPEK. 1902. Beitr. z. chem. Phys. u. Path. 1, 538; 2, 557; 3, 47.
DARWIN, C. 1876. Insektenfressende Pflanzen (German edition by Carus). Stuttgart.
DIAKONOW. 1887. Ber. d. bot. Gesell. 5, 380.
DUCLAUX. 1885. Compt. rend. Soc. biolog.
DUCLAUX. 1889. Annales Instit. Pasteur, 3, 97 and 413.
ESCHENHAGEN. 1889. Einfl. v. Lösungen versch. Konz. auf Schimmelpilze. Diss. Leipzig.
FERMI and BUSCAGLIONI. 1899. Centrbl. Bakt. II, 5, 24.
FISCHER, ALFR. 1897. Vorlesungen über Bakterien. Jena.
FISCHER. 1903. Ibid., 2nd ed. Jena.
GOEBEL. 1891-93. Pflanzenbiol. Schilderungen. Marburg.
HEINRICHER. 1895. Beitr. zur Biol. d. Pfl. 7, 315.
HEINRICHER. 1897, onwards. Jahrb. f. wiss. Bot. 31, 77; 32, 389; 36, 665; 37, 264.
HEINSIUS V. MAYENBURG. 1901. Jahrb. f. wiss. Bot. 36, 381.

Jensen, Hj. 1898. Centrbl. Bakt. II, 4, 401.
Jost. 1895. Jahrb. f. wiss. Bot. 27, 403.
Katz. 1898. Jahrb. f. wiss. Bot. 31, 599.
Koch, L. 1880. Die Klee- u. Flachsseide. Heidelberg.
Koch, L. 1887. Entwicklungsgesch. d. Orobanchen. Heidelberg.
Koch, L. 1889. Jahrb. f. wiss. Bot. 20, 1.
Koch, L. 1891. Ibid. 22, 1.
Kohnstamm. 1901. Bot. Centrbl. (Beihefte) 10, 90.
Laborde. 1897. Annales Instit. Pasteur, 11, 1.
Laurent. 1889. Annales Instit. Pasteur, 3, 368.
Laurent. 1890. Koch's Jahresb. über Gärungsorg. 1, 54.
Laurent. 1898. Compt. rend. 127, 786.
[Laurent. 1904. Revue gén. de Bot. 16, 14.]
Malfitano. 1900. Annales Instit. Pasteur, 14, 60 and 240.
Meissner, Curt. 1902. Akkommodationsfähigkeit d. Schimmelpilze. Diss. Leipzig.
Meissner, R. 1900. Centrbl. Bakt. II, 6.
[Molisch. 1893. Sitzb. Wien. Akad. 102, I, 423.]
Nägeli. 1879. Ernährung d. nied. Pilze. Bot. Mitt. 3, 395.
Nägeli. 1882. Unters. über nied. Pilze. Munich and Leipzig.
Nikitinski. 1902. Jahrb. f. wiss. Bot. 37, 365.
Pasteur. 1858–60. Compt. rend. 46, 617 ; 51, 298.
Pasteur. 1860. Annales Chim. et Phys. III, 58, 323.
Pasteur. 1862. Ibid. III, 64, 106.
Peirce. 1894. Annals of Bot. 8, 53.
Pfeffer. 1895. Jahrb. f. wiss. Bot. 28, 205.
Pfeffer. 1896. Ber. Sächs. Gesell. Wiss. (Math.-phys. Cl.), 513.
Pitra. 1861. Bot. Ztg. 19, 53.
[Raciborski. 1905. Bull. Acad. de Cracovie, Math.-nat. Cl. 461.]
Raulin. 1869. Annal. Sc. nat. v, 11, 91.
Reinitzer. 1900. Bot. Ztg. 58, 59.
Reinke. 1883. Unters. aus d. bot. Labor. Göttingen, 3, 13.
Sanguinetti. 1897. Annal. Instit. Pasteur, 11, 264.
Thiele. 1896. Temperaturgrenzen d. Schimmelpilze (comp. Pfeffer, Phys. I, 373 .
Vines. 1897–1902. Annals of Botany, 11, 563 ; 12, 545 ; 15, 563 ; 16, 1.
Wehmer. 1895. Beitr. z. Kenntnis einh. Pilze, Jena, Heft 11, p. 86 (Koch s Jahresbericht, 1895).
Went. 1901. Jahrb. f. wiss. Bot. 36, 611 (also Centrbl. Bakt. II, 8, 544).
Winogradsky. 1899. Centrbl. Bakt. II, 5, 342.
Wortmann. 1882. Zeitschr. f. physiol. Chemie, 6, 287.
Zumstein. 1899. Jahrb. f. wiss. Bot. 34, 149.

LECTURE XVI

RESPIRATION

So far we have been studying *certain* of the chemical processes which go on in the plant from one point of view only ; we have dealt with the phenomena of *assimilation*, i. e. the construction of *complex* compounds out of *simple* ones, of *organic* substances out of *inorganic*, and also glanced at the alterations which products of constructive metabolism undergo when they become reserves, plasta, &c. ; but we drew special attention to the fact (p. 124) that another series of processes took place in the plant which resulted once more in the formation of simple bodies from complex. This statement we must now emphasize. Throughout the *entire* plant and *at all times* what may be termed *dissimilation* is going on—a process which partly, at least, undoes what assimilation has done. On examining a leaf which has been assimilating all day, but which has been prevented from getting rid of the products of assimilation we find that it does not contain at nightfall *as much* carbon in the organic form as we should expect from the amount of carbon-dioxide decomposed, nor does the plant as a whole

at the end of summer contain as much organic material as would be equivalent to the sum of the amounts assimilated during the several days of the annual period of metabolic activity. The difference between the total amount assimilated and the total amount dissimilated is the increase in dry weight—the net result of normal plant growth. Plants may be easily cultivated under conditions where assimilation is prevented or greatly retarded (e. g. in the case of autotrophic plants grown in darkness, or of heterotrophic plants in absence of nutrients), and under such circumstances destructive metabolism still goes on and continued growth now results in a *diminution in dry weight.*

This is admirably shown by a study of seedlings which have been grown in the dark, though at first sight such a conclusion is by no means obvious. The seedlings grow from day to day, and roots and shoots increase markedly in volume, but that increase is entirely due to absorption of water, and the dry weight, and more especially the organic material, decreases daily. The following summary of one of BOUSSINGAULT'S experiments shows this clearly (DETMER, 1880, p. 247) :—

Material.	Dry weight of seeds.	Dry weight of seedlings several weeks old, grown in darkness.	Loss.
46 wheat grains	1·665 gr.	0·713 gr.	0·952 gr.
10 peas	2·237 gr.	1·076 gr.	1·161 gr.

A comparison between assimilation and dissimilation is more readily made in a fungus than in one of the higher plants, for we have only to determine how much nutritive material (e. g. sugar) the fungus has absorbed, how much dry substance it has formed from it, and how much it might have formed. As a basis for this last calculation we reckon that a fungus can construct about 2 gr. of dry weight for every gram of cane sugar absorbed, instead of which we find that only 0·4 gr., or 0·5 gr., or even less, is produced. PFEFFER (1895, 257) and KUNSTMANN (1895) have termed the numerical relation between the sugar used up and the fungal substance formed the 'economic coefficient'. Theoretically the minimum value of this coefficient is about $\frac{1}{2}$, but in reality it has always been found to be greater than unity. KUNSTMANN (1885) gives it as from 1·13 to 3·38, and ONO (1900) obtained a value as high as 6·1, so that we must not consider the coefficient as in any sense a constant, nor look on the plant as always an economical worker. The coefficient increases, for example, with the progressive development of the fungus and with elevation of temperature. Among other external influences poisons must be specially noted, the stimulating effects of which in weak doses we have already drawn attention to. Thus it has been shown by ONO that the addition of a 0·003 per cent. to a 0·03 per cent. solution of zinc sulphate reduced the economic coefficient, in the case of *Aspergillus*, from 6 (or 4 in other experiments) to about 2·8. The chemical stimulus resulting from the addition of such substances induces an economy in the expenditure of food materials. The deficit appearing from day to day under all conditions is the result of *dissimilation.*

No organism can remain in existence without constantly losing weight from the dissimilation or destruction of organic substance. We may term this katabolic process *respiration,* whatever be the nature of the final products, or we may reserve this name for those destructive changes which are exhibited by most plants under ordinary conditions and which result in the formation of carbon-dioxide and water from organic materials. Of these products of decomposition the most obvious one is carbon-dioxide; the production of water is much less easily demonstrated. Both carbon-dioxide and water may be obtained by decomposition of organic materials such as starch, sugar, &c., not merely in the course of respiration in the organism but also by ordinary combustion. Hence we may conclude that respiration is a combustion process, and every experiment goes to show

that *oxygen* is essential to the maintenance of respiration. Respiration may, therefore, be also described as an oxidation process, standing in marked contrast to carbon assimilation, which we have learned to regard as a process of reduction.

It will be necessary for us to study, in the first instance, the *methods of demonstrating respiration* so that we may appreciate to what extent respiration occurs in the vegetable kingdom. As a proof of its occurrence we shall employ, as a general rule, the excretion of carbon-dioxide, a gas which may be measured quantitatively and qualitatively without any difficulty. Place, for example, a handful of germinating seeds in a flask, closed by means of a rubber stopper through which passes a glass tube, and keep the tube closed for a few hours; then open the tube under lime water—the resulting cloudiness in the fluid demonstrates that a certain amount of carbon-dioxide was present in the flask. In place of lime water caustic potash may be employed, as this substance readily absorbs carbon-dioxide and replaces the gas in the flask by way of the glass tube. Perhaps the simplest, although a more indirect, method of all for demonstrating respiration is based on the fact that oxygen is used up in proportion to the carbon-dioxide given off. If we place some germinating seeds, young leaves, buds, &c., at the bottom of a tall glass cylinder closed by a stopper, and if after several hours we insert a burning taper into the jar after careful removal of the stopper, we shall see from its immediate extinction that the enclosed air has been deprived of most of its oxygen through the activity of the vegetable structures within.

It is obvious that both the method by absorption of carbon-dioxide by caustic potash and that by precipitation of carbonate of lime on the addition of lime water furnish us with a means of determining quantitatively how much carbon-dioxide is produced during respiration, still the use of completely closed spaces in such experiments is to be avoided, since respiration itself, under such conditions, becomes abnormal owing to the rapid decrease in the amount of oxygen present. It is preferable, therefore, to place the plant experimented on in a vessel through which a continuous stream of air may be driven. This air is deprived of all its carbon-dioxide before entering the vessel and becomes once more charged with that gas within it; the amount added may be easily determined as the gas leaves the vessel at the other end. Into the purely chemical details of the experimental method it is unnecessary for us to enter. The amount of oxygen used up may also be employed as a measure of the amount of respiration taking place, as well as the amount of carbon-dioxide produced.

The first conclusion we arrive at from a study of comparative estimates of the intensity of respiration is that different plants, different members of the same species, and even the same organ of an individual plant in different stages of development exhibit the widest possible variations. Certain biological groups, such as oily and shade-loving plants, are remarkable for the feebleness of their respiration, while on the other hand, many Fungi exceed the warm-blooded animals in respiratory activity. Flowers, embryonic organs, germinating seeds, buds, &c., appear to respire more vigorously than full-grown roots, stems, or leaves, assuming of course that external conditions remain constant. It will be advisable at this stage to illustrate these statements by a few tables.

According to AUBERT (1892, p. 375) the following plants absorb hourly the following amounts of oxygen (in ccm.) per gram of fresh weight, at a temperature of 12° to 15° C. :—

Cereus macrogonus	3·00	*Picea excelsa*	44·10
Opuntia cylindrica	6·80	*Lupinus albus*	73·70
Opuntia maxima	15·30	*Tulipa europea*	89·60
Phyllocactus grandiflorus	28·70	*Faba vulgaris*	96·60
Sedum album	56·60	*Mirabilis jalapa*	120·00
Sedum acre	72·45	*Triticum sativum*	291·00

The succulent plants in the first series, it will be noted, respire on an average far less actively than the others.

As examples of excessive respiratory activity, GARREAU'S (1851) data, obtained from germinating seeds, may be next quoted :—

Plant.	Temperature.	Fresh weight of seeds.	Dry weight of seeds.	CO_2 in 24 hrs.	CO_2 per 1 gr. of dry weight.
Lactuca sativa	16° C.	4·5 gr.	0·40 gr.	33 ccm.	82·5 ccm.
Valerianella olitoria	,,	4·0 ,,	0·20 ,,	25 ,,	125 ,,
Papaver somniferum	,,	5·8 ,,	0·45 ,,	55 ,,	122 ,,
Sinapis nigra	,,	8·5 ,,	0·55 ,,	32 ,,	58 ,,
Lepidium sativum	,,	2·5 ,,	0·25 ,,	12 ,,	48 ,,

The same investigator gives the following values for buds :—

Plant.	Temperature.	Fresh weight of buds.	Dry weight of buds.	CO_2 in 24 hrs.	CO_2 per 1 gr. of dry weight.
Syringa	15° C.	9·0 gr.	2·0 gr.	70 ccm.	35 ccm.
Sambucus nigra	,,	10·0 ,,	1·75 ,,	60 ,,	34 ,,
Ribes nigrum	,,	7·0 ,,	1·25 ,,	60 ,,	48 ,,
Tilia europea	,,	4·0 ,,	0·70 ,,	46 ,,	66 ,,

These numbers cannot be directly compared with those obtained by SAUSSURE (1804) (compare SACHS, 1865, p. 277) for flowers and floral organs, because that author estimated the volumes of oxygen absorbed and reckoned the volume of the organ in question as unity. His results are, all the same, of great interest because he investigated not only the flowers but also the *leaves* of the same plants in darkness.

Plant.	Oxygen used up by flowers in 24 hrs.	Oxygen used up by the reproductive organs in 24 hrs.	Oxygen used up by the foliage leaves in 24 hrs.
Cheiranthus cheiri	11·0	18·0	4·0
Polyanthus tuberosus	9·0	—	3·0
Tropaeolum majus	8·5	16·3	8·3
Passiflora serratifolia	18·5	—	5·25
Cucurbita melopepo ♂	7·6	11—7 (anthers)	—
Cucurbita melopepo ♀	3·5	4—7 (stigmas)	—
Ilex aquifolium	—	—	0·86
Viburnum tinus	—	—	2·23
Juglans regia	—	—	4·4
Populus alba	—	—	4—6

Finally, a few examples may be quoted by way of demonstrating the variations which occur *during development*, and the first of these is of interest as giving the absolute amount of respiration. The inflorescence of *Arum* used up the following amounts of oxygen (in ccm.) in successive hours (GARREAU, 1851) :—

	Experiment 1.	Experiment 2.	Experiment 3.
1st hour	39	75	45
2nd ,,	57	95	70
3rd ,,	75	125	95
4th ,,	100	85	140
5th ,,	50	55	85
6th ,,	20	25	35
Total	341	460	470
In 18 following hours	184	230	300

If we express these results graphically we obtain a curve similar to that obtained for many other physiological processes. Fig. 36 represents such a curve, based on RISCHAVI'S experiments on germinating wheat, where the abscissae represent days and the ordinates the amounts of carbon-dioxide produced daily in mg.

The examples quoted above furnish us with an approximate estimate of the variations in respiration but are not adapted to exact comparative study, because in some cases the oxygen absorbed is determined, in others the carbon-dioxide produced, the calculations being based either on volume or on weight and because in some cases the fresh weight, in other cases the dry weight or the volume of the parts are used to found estimates on. Strictly speaking none of these methods are quite accurate, for we shall see that it is the living protoplasm that is really the seat of respiration. The point of special interest is to determine whether or not differences exist in the amount of respiration taking place in the protoplasm of these organs, but unfortunately we have no data as to the amount of protoplasm, either volumetric or gravimetric, to serve as starting-points for such a comparison. All we know is that the amount of protoplasm present in young organs is relatively much greater than in mature organs, and that so far as we at present are aware, the respiratory variations at different developmental stages may be thus, at least partly, accounted for. It is, however, also extremely probable that a given amount of protoplasm may respire with varying intensity according to its condition. It may be sufficient at present to draw attention to the two chief vital conditions in which protoplasm occurs, viz. the active and the passive, the former being exhibited during the vegetative period, the latter during the summer or winter resting period. Under constant external conditions the resting protoplasm of tubers, bulbs, trees, &c, exhibits marked differences to the same protoplasm in the active state, evidenced by the greatly diminished intensity of respiration, but so long as the necessary external factors are present no protoplasm entirely ceases to breathe.

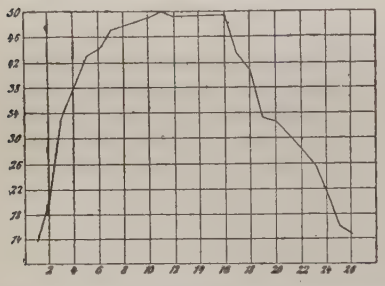


Fig. 36. Curve of carbon-dioxide excretion (in mg.) of 40 wheat-seedlings at a temperature of 21° C. After RISCHAVI (1876).

Proof of this continuous respiration is not always easy to establish since it may be completely masked by other processes. Thus, as we have already seen, cells containing chlorophyll decompose carbon-dioxide in sunlight. Such cells, even though they be respiring can still give off oxygen, or may, if respiration and assimilation be equally active, fail to show any evidence of gaseous exchange. In fact as the light decreases in intensity the amount of oxygen given off also decreases; later on, it ceases altogether, and finally an evolution of carbon-dioxide manifests itself instead. This is, doubtless, most simply explained by assuming that respiration and assimilation go on concomitantly and quite independently of each other. Although there are no good reasons for assuming that reduction and oxidation go on in the same cell, yet it is very difficult to prove this exactly, since it is possible that respiration, which can be easily demonstrated to occur in a green leaf in the dark, is masked when the leaf is exposed to light. Observations made on non-green tissues and organisms do not aid us much in this relation. For long, attempts have been made to study these two antagonistic functions separately and not without a certain amount of success. CL. BERNARD (1878) was the first to inhibit assimilation by means of chloroform vapour, and AD. MAYER (1879) noted that a similar result might be obtained by using prussic acid. It has been shown generally that assimilation may

be inhibited more readily than the *respiratory function* by the employment of such poisons; for EWART (1896) was able to inhibit assimilation for a certain time by the use of ether vapour, although respiration continued and the organism remained alive. Such experiments would, however, have increased value if it were possible by etherization to inhibit assimilation completely whilst leaving respiration entirely unaffected. BONNIER and MANGIN (1886) have attempted to do this, but their results, taken in relation with those of other observers, are open to criticism, and may indeed have been obtained rather by good fortune than otherwise. It has often been observed that weak etherization accelerates respiration (ELFVING, 1886; JOHANNSEN, 1896; MORKOWIN, 1899), whilst strong etherization, by killing the cells, inhibits it. That respiration may continue constant in leaves placed in a narcotic condition is possible, but, as we have said, it is only by chance that such a result is obtained, and experiments carried out with leaves of the same kind do not confirm BONNIER and MANGIN'S results.

Another method of proving directly that respiration takes place in green cells exposed to light, which was for long believed to be effective, was that employed by GARREAU (1851). GARREAU showed (by means of baryta water) that demonstrable traces of carbon-dioxide always escaped from illuminated branches, and he believed he was dealing in that case with carbon-dioxide formed in the process of respiration and escaping from the chloroplasts. BLACKMAN (1895) showed, on the other hand, that this excretion of carbon-dioxide was very improbable, and that it was incapable of demonstration if one experimented exclusively with chlorophyll-containing cells and rejected all peduncles and stems not possessing green colour, a precaution which GARREAU failed to take.

Although direct proof of continuous respiration in illuminated green cells cannot be obtained, indirect evidence is available. We may observe not infrequently in green cells during active assimilation a continuance of protoplasmic movement and growth, two phenomena which are impossible in the absence of respiration. In general, it is correct to assume that respiration takes place in light to as great an extent as in darkness. The assimilatory activity of a foliage leaf must be estimated not only by direct measurement of the amount of carbon-dioxide taken up from without but also by estimating the amount of carbon-dioxide which is produced during the same time by respiration, but which does not escape from the plant because it is at once employed in assimilation (compare Lecture X, p. 124).

Since, also, the *respiratory* gaseous exchange is, under normal conditions, far less intense than the *assimilatory* gaseous exchange, it follows that in the long run a plant organ may suffer from scarcity of oxygen or from the presence of injurious quantities of carbon-dioxide. Carbon-dioxide, when in sufficient accumulation, undoubtedly interferes with the essential functions of the plant, so that removal of the gases arising in the course of respiration may be considered as absolutely necessary. The removal of these is a simple matter in the assimilating leaf. If an accumulation of carbon-dioxide has taken place in it during the night, in the morning it is at once removed on the commencement of assimilation. The abundant intercellular spaces with their openings, the stomata, are well adapted for the promotion of thorough aeration. The gaseous exchange is conducted with greater difficulty in colourless subterranean organs; but here also the individual cells, by means of intercellular spaces, are, generally speaking, favourably situated for giving off and taking up gases. Since these organs, however, have no direct exit passages the gases must either travel long distances to reach the aerial stomata or must escape by diffusion through the cuticle. Without doubt the cuticle of subterranean organs offers much less opposition to such a diffusion than does that of the foliage leaf; we have already seen how permeable it is to water, and it would appear to be equally permeable to carbon-dioxide. So far also as the oxygen is concerned, that gas, owing to vigorous partial pressure,

will easily pass through the outer walls of the cells. The matter is not so certain in the case of water plants because the oxygen surrounding them has only a low tension. An investigation of the intercellular space system in all plants, whether aerial, subterranean, or submerged, teaches us that accumulation of carbon-dioxide and deficiency in oxygen never reach a degree worth considering, and hence that the means at the disposal of the plant are always sufficient for maintaining a gaseous exchange. Carbon-dioxide to the extent of 5 per cent. and oxygen as low as 8 per cent. are only seldom met with in intercellular spaces, and PFEFFER and CELAKOWSKI have shown that in the interior of individual cells oxygen is never wanting. PFEFFER (1889) studied Rotifera living in the cell-sap of *Vaucheria*, which moved actively under normal conditions, but whose movements ceased when oxygen is prevented from entering. CELAKOWSKI (1892) studied the protoplasmic streaming in cells of *Tradescantia* which had been absorbed by the plasmodium of a Myxomycete, and found that these movements continued inside the plasmodium; the cells must, therefore, have been abundantly supplied with oxygen.

Having now gained some acquaintance with the general occurrence of respiration we have to inquire next as to the substances which undergo respiration and the products resulting therefrom, products which naturally are related to those arising from ordinary combustion. In many cases it may be shown that starch and sugar disappear during respiration. If these were completely burnt we must expect carbon-dioxide and water as the final products. From the formula

$$C_6H_{10}O_5 + 6O_2 = 6CO_2 + 5H_2O$$

it may be seen that for every volume of oxygen taken in one volume of carbon-dioxide must be produced, and in many cases the respiratory quotient $\frac{CO_2}{O_2}$ has actually been found to be unity. The contemporaneous formation of water may also be demonstrated. SAUSSURE (1804, p. 17) long ago remarked that germinating seeds lost more weight than one would expect from the amount of carbon-dioxide formed, and he thought that this was due to loss of water 'which previously was united with the substance of the seed'. LASKOWSKY (1874) investigated the origin of the water by exact methods and found it to be *about* equal to the amount which was to be expected from the formula given above.

It would be quite wrong to expect unity as the value of the fraction $\frac{CO_2}{O_2}$ in higher plants, on the assumption that carbohydrates were exclusively used up in respiration. The *average* value for the fraction is the result of a number of processes, each of which varies from unity, being sometimes greater, sometimes less.

The higher plants do not lend themselves readily to such experiments, because it is difficult to say in most cases what substances are undergoing combustion. It is quite otherwise with Fungi; in their case we have it in our power to supply the organism sometimes with one kind, sometimes with another kind of material. On this subject we owe much to PURIEWITSCH'S (1900) thorough researches on *Aspergillus*, and the following table gives a summary of his results :—

Relation of Carbon-dioxide to Oxygen in *Aspergillus*.

Nutrient.	1 %	1·5–2 %	3 %	5 %	10 %	15–17 %	20–25 %
Dextrose	0·9	0·9	—	1·06	1·18	0·73	—
Cane sugar	0·87	—	—	0·96	1·02	—	0·83
Raffinose	0·91	—	0·66	—	—	—	—
Starch	0·68	0·55	—	—	—	—	—
Glycerine	—	0·77	—	0·78	0·69	—	—
Mannite	0·66	—	—	0·49	0·65	—	—
Tannin	0·91	—	—	0·50	0·43	—	—
Tartaric acid	—	1·59	1·52	1·78	1·6 (7 %)	—	—
Lactic acid	0·69	0·89	0·98 (4 %)	—	—	—	—

It is not possible to deduce from these experiments any regular dependence of the quotient on the quantity or constitution of the material used up in respiration, still these results are full of interest as showing how extraordinarily variable the quotient is, whose value in the majority of higher plants examined—the exceptions we have still to study—lies close to 1. This fact, also established by PURIEWITSCH, is especially important, viz. that in an individual experiment with a definite mycelium, which was placed in various nutritive solutions in succession, the fluctuations in the amount of carbon-dioxide excreted run by no means parallel with those in the amount of oxygen taken in (average values are given above); while the fluctuations in the oxygen absorbed were limited (up to 35 per cent.), those of the carbon-dioxide excreted varied within far wider limits (28 to 120 per cent.). The two processes which in chemical combustion follow each other so closely that we may consider them as simultaneous, are frequently in physiological combustion widely separated. Physiological combustion is by no means a simple process, many intermediate reactions lie between the absorption of oxygen and the excretion of carbon-dioxide, and these vary under different conditions. As a rule, the quotient $\frac{CO_2}{O^2}$ is less in value than unity, some oxygen being stored in the plant, so that we may conclude that the final products of the combustion of respiratory material are not in this case carbon-dioxide and water, but are, at least *in part*, other bodies as well, and *organic acids* suggest themselves at once to us as probable products, more especially as the occurrence of these bodies in Fungi has long been known.

Oxalic acid is very frequently formed, and an exhaustive study by WEHMER (1891) has made us acquainted with the details of its manufacture. Among Fungi, *Aspergillus niger* is known to form oxalic acid in great quantity, and WEHMER'S experiments were carried out mainly on this plant. The chief results which he obtained are summarized in the following table :—

Nutrient. 1·5 g. in each case.	Weight of the fungus.	Weight of the oxalic acid formed, estimated as a calcium salt.
Tartaric acid	0·155 g.	0·00
Citric acid	0·240 g.	0·00
Ammonium tartrate	0·030 g.	0·767
Potassium tartrate	0·032 g.	0·550
Ammonium citrate	0·056 g.	0·390
Dextrose	0·228 g.	0·278

It appears that the formation of the acid is not necessarily connected with the growth of the fungus; it arises only when the substratum gives *no acid* reaction and when the fungus is cultivated in sugar, proteid, glycerine, oil, and salts of organic acids. No oxalic acid is formed if the nutritive substance be a *free* acid, and the addition of phosphoric or hydrochloric acid to the nutritive solution inhibits its formation. More recently, WEHMER (1897), and also EMMERLING (1903), have obtained other results with *Aspergillus niger*, so that probably there are several physiological forms of this fungus. The formation of oxalic acid in *Aspergillus* has probably only a *biological* significance. The fungus grows well in an acid substratum, and if the substratum be not acid it makes it so, so that associated organisms are excluded. A continuous formation of acid would in the long run prove injurious to *Aspergillus* itself; as a matter of fact, it ceases to produce any more when the substratum contains above 0·3 per cent., but if the acid formed be neutralized the fungus can be made to produce more. In one experiment, for example, 1·253 g. of anhydrous oxalic acid was produced from 1·5 g. of sugar, whilst 2·25 g. might have been produced by its complete transformation; the 1·5 g. of sugar was altered as follows :—0·8318 g. was oxidized into oxalic acid, 0·290 g. was employed in the architecture of the fungus, and the remainder (0·3782 g.) oxidized into carbon-dioxide.

In a corresponding experiment with *ammonium tartrate* only about half the amount of oxalic acid possible was formed, whilst *free tartaric* acid was completely oxidized into carbon-dioxide and water. Since, however, it is obvious that the respiratory material is used up to a considerably less extent in an incomplete oxidation such as that which takes place when oxalic acid is formed than when carbon-dioxide is produced, it becomes a question whether this incomplete utilization of respiratory material does not make itself felt in the growth of the fungus. The increase in the dry weight of the fungus is, however, the same whether oxalic acid be formed or not; the oxalic acid lost has no great nutritive or respiratory value (compare Lecture XVII). Further, under certain conditions, the oxalic acid formed may be still further used for respiratory purposes by the fungus itself. [WEHMER (1891, a) shows that at high temperatures (above 30° C.) the oxalic acid is *always* oxidized by the fungus.]

Just as *Aspergillus* (and *Penicillium*) form oxalic acid, so *Citromyces* (WEHMER, 1894) manufactures citric acid and can itself make use of it again. *Citromyces glaber* can acidulate its substratum up to 4 per cent. of citric acid; it can withstand as much as 20 per cent. of citric acid, although it is very sensitive to the presence of inorganic acids.

In addition to proving that the formation of the acid is useful to the fungus in acidifying its substratum, WEHMER's research is also specially important as showing that the formation of the acid is not due to a *deficiency of oxygen*, as was formerly thought to be the case in this and other instances.

Indeed a formation of acid takes place in almost all plants, and although this occasionally perhaps occurs during the process of synthesis it is associated, for the most part, with the process of respiration. BENECKE (1903, Bot. Ztg. 61, 79) has shown clearly that in the higher plants also, where it is very widely distributed, oxalic acid is produced under the same conditions as in Fungi. Since it is possible to grow certain plants, such as maize, both with and without oxalates it is manifest that here also oxalic acid is not an essential product of metabolism. Generally speaking, however, the formation of organic acids during respiration is quantitatively less than that of carbon-dioxide, and it is only amongst *succulents* that one finds organic acids produced in such large quantities that the formation of carbon-dioxide is, at least at first, completely inhibited. It has long been known that the amount of acid in the leaves of these plants increased greatly at night, but it is to the comprehensive researches of AD. MAYER (1875–87), G. KRAUS (1886), WARBURG (1886) and AUBERT (1892), that we owe a knowledge of the details of the case. In darkness these plants absorb oxygen without giving off equal quantities of carbon-dioxide; the atmosphere surrounding them decreases in volume. Malic acid occurs in the Cactaceae, isomalic acid in the Crassulaceae, oxalic acid in the Mesembryanthemaceae, and the formation of the acid takes place so freely that one may detect its presence by tasting the leaves. The extreme case is when $\frac{CO_2}{O_2} = 0$, that is to say, when no carbon-dioxide is formed at all. In continued darkness and at higher temperatures the coefficient increases in value but never reaches unity. Continued formation of these acids would lead to serious injury, which the plant avoids by gradually having recourse to normal respiration and the formation of carbon-dioxide when a certain limit has been reached. This fact shows that we have here to deal with a special peculiarity of succulents, and that the formation of acids cannot depend upon an insufficiency of oxygen. Possibly, as in the case of Fungi, succulents may obtain certain advantages from the formation of acid: that is indeed true, but the purpose of the formation of the acids is naturally very different in the two cases. The acids break down in sunlight, not only when they are exposed in pure solutions, but also more especially in the presence of certain accelerating bodies which act catalytically; then carbon-

dioxide is formed which can at once be assimilated. While in ordinary plants the respiratory products escape from the plant, in the case of succulents they are retained in the leaves and carbon-dioxide arises just at the moment when it may be again used up. Obviously it is a matter of great difficulty to provide fleshy leaves with carbon-dioxide from the air. The acquisition of carbon-dioxide depends on the existence of wide-open stomata and abundant intercellular spaces, and these are features which accelerate transpiration; but the succulents live under conditions which forbid copious transpiration, and hence they do not possess these adaptations for vigorous gaseous exchange. We need only refer in a word to the fact that these leaves were shown in the lecture on carbon assimilation also to be peculiar, since one can, on conceivable grounds, establish in their case a value for $\frac{CO_2}{O_2}$ in assimilation, which varies very considerably from the ordinary (p. 110). In the morning, at the commencement of assimilation, the leaves give off far more oxygen than they absorb carbon-dioxide, indeed they can continue to give off oxygen in an atmosphere free from carbon-dioxide as long as the carbon-dioxide arising from their own activity is at their disposal.

In succulent plants, as in Mould-fungi, there are special features connected with the formation of acids which are not to be explained from the chemico-physiological point of view. The formation of acids has the same *general* significance as the complete combustion of organic materials attained elsewhere, but it has a *subsidiary biological* meaning differing widely from normal respiration. There are many down-grade metabolic products of general occurrence in plants which serve ecological purposes only, and it is quite likely that organic acids are similarly to a certain extent, regulatory, in order to render turgidity of cells possible. For example, in the formation of oxalic acid from glucose a convenient medium is produced capable of inducing osmotic pressure in the cell up to three times the normal.

Let us now return to the discussion of the value of the respiratory fraction $\frac{CO_2}{O_2}$. We have seen that variations from its typical value ($= 1$) might be the result of the formation of unusual respiratory *products*; but these variations may also be due to the varying constitution of the *materials* employed in respiration.

We have already established the fact that large quantities of fat are stored in many seeds, materials which are very much poorer in oxygen than carbohydrates. When these seeds germinate the fats undergo combustion, and SAUSSURE showed long ago that during the process an absorption of oxygen took place which does not correspond to the amount of carbon-dioxide given off; the quotient $\frac{CO_2}{O_2}$ is less than unity. BONNIER and MANGIN (1884) found, for example, in *Linum*, the following values for $\frac{CO_2}{O_2}$ on successive days; 0·30, 0·34, 0·39, 0·40, 0·63, 0·64. The vigorous absorption of oxygen, leading to an *increase* in the dry weight (DETMER, 1880, 335) takes place especially in the first days of germination; later on, when the fats are gradually altered into carbohydrates the value of $\frac{CO_2}{O_2}$ gradually approaches unity. He observed, for example, that in a seedling 3·5 cm. in length the value of the fraction was 0·81, an amount as great as that seen only in plants which are beyond the seedling stage (e. g. in *Pinus*; BONNIER, 1884, p. 240).

On the other hand, when fatty oils are formed from carbohydrates in ripening seeds, an increase in the respiratory coefficient is naturally to be expected:

as a matter of fact GERBER (1900) found it to be as much as 4·71 in *Ricinus*, i. e. almost five times as much carbon-dioxide was formed as oxygen absorbed.

The fact that sugar may be formed by oxidation in destructive metabolism is worthy of special attention, because it shows better than any other illustration that a physiological classification of materials cannot be made to agree with a chemical classification, since one and the same substance, in the present instance sugar, can constitute at once a product of assimilation in constructive metabolism and a respiratory product in destructive metabolism. Further, we do not always meet with sugar in the germination of oily seeds, as for example, in the onion; it is wanting in *Cannabis*, where it is true it is formed but very quickly changed into starch. Starch and sugar serve later on equally well as supporters of respiration and as constructive materials for the manufacture of cell-walls.

Carbohydrates and fats can to a certain extent replace each other in the plant as respiratory material. This is known to be the case also in the animal world, but there the two substances are not sufficient to maintain life, which is dependent on a constant destruction and oxidation of proteid, followed by the excretion of such nitrogenous waste as hippuric acid, urea and uric acid. The question thus arises whether proteid is also destroyed in the plant during respiration and whether that takes place *of necessity*. It may be easily shown that peptone can act as a respiratory material, in Fungi especially, and it will be sufficient to quote here an experiment of WEHMER'S (1892) on *Aspergillus*. This fungus thrives remarkably well on peptone as a source of nitrogen and sugar as a source of carbon, but there are no obvious indications that the peptone is used as respiratory material also. The fungus is, however, able to supply all its wants both as to carbon and nitrogen when peptone *alone* is supplied to it. Under these circumstances functions carried out by sugar must be undertaken by the peptone, and it appears that a part of the nitrogen of the peptone is transformed into ammonia and excreted. The insight we thus obtain into the process of respiration is only somewhat limited, since the nitrogen of the ammonia produced in this reduction cannot be compared with the carbon of the carbon-dioxide produced in the oxidation of carbohydrates. Ammonia is a *secondary product* of peptone respiration, which comes off free when the carbon of the peptone is turned into carbon-dioxide in the process of combustion. The appearance of products of reduction in respiration will be dealt with later, at present we need only note how extremely varied a mould fungus can be in its metabolism: ammonia, the same material which in combination with sugar serves as a source of nitrogen, is also given off as a worthless waste product when peptone is supplied as the single organic nutrient.

The other question, as to whether some proteid *must always* be respired, is not so easily answered. This was more often referred to the decomposition of proteid especially noticeable in seedlings but which has been clearly demonstrated elsewhere and which leads to the formation of amido-compounds. We have considered these bodies as products of the hydrolytic decomposition of proteid, and have assumed that the formation of these crystallizable and easily diffusible substances was necessary, since proteid as such is not well adapted to translocation through the plant body. It is quite possible, however, that the amido-compounds arise in respiratory metabolism and are produced from proteids by oxidation. This view, at the present moment, can neither be proved nor disproved, still when the condition of things in animals is taken into account we must regard it in a certain sense as probable. It is easily understood, according to the earlier statements made, that the products of the decomposition of proteid in plants are not excreted. The amido-compounds which are formed under suitable conditions are capable of being again made use of for the regeneration of proteid; and this fact increases the difficulty of completely solving this problem. If the amido-

compounds were indeed the final products of respiratory metabolism, we might say that the green plant differs essentially from the higher animal in being able to make use both of its nitrogenous and non-nitrogenous metabolic products as nutritive materials once more. Since animals do not possess this power, they may in that respect be contrasted with plants.

Respiration, like all other functions of the organism, depends on the external factors of which we have already had occasion to speak. *Light*, according to KOLKWITZ (1899), produces a feeble rise in respiration—at least in Fungi—although it is impossible to say whether its action is purely chemical (by decomposition of certain organic acids) or whether its influence is more far reaching and affects the protoplasm. Since in other cases, however, a reduction in the respiration has been observed when the plant is illuminated, the question cannot be considered as in any way settled. More recently, MAXIMOW (1902) has only partially confirmed the results obtained by KOLKWITZ. One thing at least is certain, that light has *no essential* influence on respiration ; *heat*, on the other hand, is of *fundamental* importance. Although the curves expressive of the dependence of the majority of physiological processes on temperature resemble very closely that of assimilation (p. 124), exhibiting between the minimum and maximum a pronounced optimum, this last datum is not quite determined for respiration. PFEFFER holds the view that increase in the intensity of respiration is concomitant with increase of temperature until the latter begins to influence injuriously all the vital processes. The plant must be permanently injured by temperatures which bring about a diminution of respiration. If, however, we accept the results arrived at by ZIEGENBEIN (1893) we must acknowledge the probable existence of an optimum temperature for respiration. ZIEGENBEIN finds that the intensity of respiration (measured by the amount of carbon-dioxide excreted (in mg.) per cent. of fresh weight) depends on the temperature in the manner illustrated by the following table :—

	10°	20°	30°	35°	40°	45°	50°	55°	60°
Potato tubers	1·17	2·22	4·62	7·85	10·24	*12·22*	11·14	10·30	2·71
Vicia faba (seedlings)	—	—	55·2	*78·72*	65·1	57·8	20·8	—	—
Abies excelsa (shoots)	—	—	185·0	*206·4*	198·4	168·9	33·3	—	—

Although one is compelled to hold that all temperatures above 45° are certainly injurious, and that the observed reduction in respiration at 50°, &c., must be regarded as the result of the plant's pathological condition owing to the excessive temperature, it is difficult to believe that a temperature of 40°, which causes a diminution of respiratory activity in the last two objects experimented on, operates in this way. ZIEGENBEIN, at least, fails to offer exact proof that this was not the case. Should an optimum temperature be established for respiration later on, it must lie in each case extremely near the maximum. Further evidence as to the optimum temperature is given by KUNSTMANN (1895) for Fungi, and STOKLASA (1903) for beetroot. The position of the minimum has been often worked out and it has been found that it lies considerably below 0° C., in the case of lichens, for example, about −10° C. (JUMELLE, 1892). The increase in respiration near the maximum temperature is often very marked ; it reaches, for example, according to CLAUSEN's experiments (1890) on germinating wheat, to as much as eleven times, and in the case of lupins to sixteen times what it is at 0° C. The fact that respiration is accompanied by a *production of heat* need only be casually referred to here, since that phenomenon will require closer consideration in another place (Lecture XXXI).

In regard to the influence of materials on respiration we may consider first of all water, which has no specific influence on respiration, but is important only in so far as it is one of the general vital conditions. Respiration ceases entirely in seeds and perfectly dry parts of plants, in mosses, lichens, &c., which can tolerate complete desiccation, continuing alive during drought without

exhibiting any obvious metabolism ; minute quantities of water enable the formation of carbon-dioxide to commence at once (KOLKWITZ, 1901). The substances which are used in respiratory metabolism are naturally of greater significance, and if they be present in insufficient quantity respiration ceases, as, for example, when plants are kept for a long time in the dark. In Fungi, such as *Aspergillus* (KOSINSKI, 1901), which accumulates no reserves, the withdrawal of the nutritive solution makes itself evident at once in a reduction of respiration ; so long, however, as the organism remains alive respiration does not cease entirely, and if nutrients are supplied to the plant after cessation of the period of starvation respiration at once increases. Under normal nutritive conditions, however, the amount of respiration is by no means proportional to the quantity of respiratory materials present, and this fact is of the utmost importance in a theoretical consideration of respiration.

Generally speaking, an increase in respiration may be noted if the plant be subjected to injurious influences. Thus, for example, small doses of certain poisons act in this way, and perhaps we may correlate this result with the increased growth which results from the addition of such stimulants (p. 88). Similar results are produced by anaesthetics and antipyretics (JACOBI, 1899), as has been already noted (p. 195). Carbon-dioxide acts in a like manner if it accumulates in excessive amounts, and the same result is produced as an after-effect of high temperatures, high atmospheric pressure, wounds, &c. (RICHARDS, 1896).

In conclusion we may note the effect of oxygen, the gas which is most concerned in respiration. It is worthy of note that respiration is independent within wide limits of the percentage of oxygen present in the air ; the partial pressure of oxygen may be reduced or increased considerably in comparison with the normal without at once influencing respiration. Hence the presence or absence of indifferent gases, such as nitrogen, appears to be without significance, respiration takes place in pure oxygen just as in ordinary air, in which the oxygen is reduced to one-fifth of its volume ; in both cases, the partial pressure of oxygen is, however, the same (i. e. one atm.) Only after the pressure of pure oxygen is raised to 2–5 atmospheres, does there ensue a marked increase in respiration followed soon after by a marked diminution until death takes place (JOHANNSEN, 1885). The fact that death always takes place under higher oxygen pressures is not due to *increase of respiration*, since far greater increase can be reached by other means, e. g. higher temperatures, without any evil effect following. Why a fatal effect should result from an increased access of oxygen we do not know ; only this much is known that the different types of plant-life behave very differently in this relation, since all transitions occur between such plants as we have as yet alone studied and organisms which become injured by pressures of oxygen far below that present in ordinary air (compare Lecture XVII).

Respiration, as already stated, is at first not affected by a reduction of oxygen pressure, and STICH (1896) showed that not until the proportion of oxygen in the air was as low as 2 per cent., or even less, was there any diminution in the amount of carbon-dioxide given off. Experiments on this question are not at all easy to carry out because, as has long been known, carbon-dioxide continues to be given off for a considerable time after the complete exclusion of oxygen. In some plants (*Vicia faba*, *Ricinus*, &c.) this excretion is not less intense than when oxygen is supplied ; in the majority of cases however it reaches only one-third to two-thirds of this value, and varies in the individual plant, according to its developmental condition. The carbon-dioxide produced in this oxygenless respiration arises from the same materials as are consumed in ordinary respiration ; still it cannot arise from simple *combustion* but from the *splitting* of organic bodies, resulting in the formation of both reduced and completely oxidized bodies. Oxygen atoms wander within the molecules of the respiratory materials in so-called intra-molecular respiration. When glucose,

for example, breaks down and all the oxygen present becomes used up in the formation of carbon-dioxide there remains over, besides carbon-dioxide, a completely reduced body composed of carbon and hydrogen; if all the oxygen be not so used up, a body poor in oxygen as compared with glucose is formed, such as alcohol, which, as a matter of fact, always appears in intra-molecular respiration, and which collects often in considerable quantities (LECHARTIER and BELLAMY, 1874, MAZÉ, 1900). Thus BREFELD (1876) found ½ per cent. of alcohol in the leaves of ivy and *Corylus* after seventeen days; in grapes, after several weeks, ½–2 per cent.; in cherries, after four weeks, 1·8–2·5 per cent.; and in pea-seedlings, after three months, as much as 5 per cent. [According to DUDE (1903, Flora, 92, 205) the plants used by BREFELD, at least partly, can withstand the withdrawal of oxygen for only a very short time (hours or days). MATRUCHOT and MOLLIARD (1903) have shown that alcohol is formed by higher plants when micro-organisms are completely excluded. In LECHARTIER and BELLAMY'S experiments Fungi or Bacteria doubtless co-operated in bringing about the result.] If seeds of *Vicia faba* are kept for two days under water one can recognize the presence of alcohol by the smell on rubbing. In addition to ethyl-alcohol other substances also appear during intra-molecular respiration, i. e. higher alcohols, acids, aromatic compounds, and even hydrogen, but as to their proportional amounts nothing is known. By means of such special decompositions of organic substance the plant parts remain long alive, the most resistant, it may be, for months, while others die in a few days or hours; the amount of carbon-dioxide produced is also, as might be expected, very variable, and in extreme cases may be seven to ten times the volume of the plant part concerned.

These last-mentioned phenomena, more especially, throw considerable light on the problem of the *factors* concerned in *respiration* [compare BARNES, 1905]. We have described respiration as a case of *combustion*, and this we were very well entitled to do from the products it gives rise to; one may arrive easily, however, at a totally incorrect conception of the causes of respiration by such a mode of expression. In ordinary combustion an oxidizable body is oxidized, during which process it absorbs the oxygen of the air, and this combustion may go on at normal or at supernormal temperatures. Physiological combustion or respiration takes place at temperatures so low that a direct union between sugar, starch, &c., and oxygen is inconceivable. Further, the oxygen cannot be the cause of the oxidation, since, necessarily, an alteration in the intensity of respiration should be observed simultaneously with an alteration in the amount of oxygen present in the cell, which, as we have already said, is not the case. Further, we have seen that respiration is within wide limits independent of the amount of materials capable of being oxidized, and hence we must also conclude that this is not the cause of physiological combustion. Should we thus be driven to assume the presence in the protoplasm of a substance which can oxidize more vigorously than ordinary oxygen, viz. the so-called 'active oxygen', we find ourselves at the same time face to face with a serious difficulty. 'Active oxygen' once present must attack *all* oxidizable bodies in the plant, whilst it is characteristic of respiration that only *some* substances are oxidized. It is difficult to see how the cell-wall could resist the attack of 'active oxygen' if sugar and starch be oxidized by it. Further, PFEFFER (1889) succeeded in bringing direct evidence against the occurrence of vigorously oxidizing substances in the cell. He showed that one might introduce into the cells of many plants dilute solutions of peroxide of hydrogen without injuring them, and that the colouring bodies occurring naturally in the plant, as well as chromogenic substances artificially introduced, suffer a change of colour which does not take place in nature.

If further proof be necessary that physiological combustion is not so simple as ordinary combustion we need only refer to the facts stated above, which show that not infrequently in respiration combustion is

only partial and reaches the formation of organic acids only and not to the final products, water and carbon-dioxide, although the amount of oxygen necessary to burn the substance completely is without doubt available. A certain light is shed on the cause of respiration by a consideration of intra-molecular respiration. If we assume with PFEFFER (1889) that normal and intra-molecular respiration have a genetic connexion, that intra-molecular succeeds normal respiration when oxygen is deficient—and this assumption is the most probable that occurs to one—then we are forced to admit the *decomposition* of organic bodies as the *primary* phenomenon in both processes. The decomposition must result in the formation of an oxidizable body, which in the presence of free oxygen takes it up, but which in its absence satisfies its requirements so far as oxygen is concerned from other compounds containing it. Carbon-dioxide must arise from it in all cases, although the intermediate products must differ according as oxygen is present or not. We must not assume that identically the same products are present in normal respiration as in intra-molecular, and that these, e. g. alcohol, become afterwards oxidized; that such an assumption cannot be correct is shown by the proportion of carbon-dioxide to oxygen in any selected case.

It is not known what the bodies are which undergo this hypothetical primary decomposition. They may be the substances which we noted disappearing in mass during active respiration, the carbohydrates; but they may be proteids or even protoplasm itself. The latter view, which has been advanced by PFLÜGER, and from the botanical side has been vigorously upheld by DETMER (1883), cannot be exactly proved, still one cannot deny its inherent probability. The abundant consumption of carbohydrates would be explained by this theory, for these would serve to regenerate the broken down proteid or protoplasm; if they be present in insufficient quantity, as in seedlings of Leguminoseae grown in the dark, the regeneration of the broken down proteid is carried only as far as asparagin; if they be entirely wanting, as in the case of a fungus nourished by peptone only (p. 200), nitrogenous loss takes place, while ammonia is formed. PFEFFER (1885, 656) has, however, advanced a serious objection to this idea, inasmuch as he has shown that *intra-molecular* respiration comes at once to an end in the absence of carbohydrates (compare DIAKONOW, 1886).

It has recently been suggested that the immediate cause of this supposed decomposition is an enzyme. HAHN (1901) believes he met with such an enzyme in the sap squeezed out of the bulbs of *Arum*, which caused sugar to disappear without the addition of oxygen. He did not isolate it, however, nor did he examine its properties in detail. On the other hand, in dead plant parts and in expressed sap *oxydases* have frequently been found, that is, bodies which act as carriers of oxygen and which colour guaiacum resin blue. That these bodies are somehow connected with respiration is not impossible, still proof must be forthcoming that they exist already in the living plant, which is certainly not the case in many. When these bodies have been more fully investigated we shall be in a better position to answer the question as to whether they are to be considered as *enzymes* and whether they are to be considered as *hydrolytic* as well as *oxidizing*. At all events, the mode of action of these two types of enzyme must be fundamentally distinct. The recent literature on the subject of oxydases includes papers by RACIBORSKI, 1898 (a criticism of whose views is given by MOLISCH, 1901; VINES, 1901); HUNGER, 1901; BEHRENS, 1901. [The literature on the subject of the occurrence and function of oxidizing enzymes in the plant has, during the past few years, increased in quantity out of all proportion to the finality and clearness of the explanations offered. We may refer to CZAPEK, Biochemie, II, 464-481, as also to CHODAT and BACH (1904) and RACIBORSKI (1905).]

Respiration is, as already stated, a process of universal occurrence in organisms, one too which is absolutely essential, since when it ceases and, generally speaking, when also oxygen is withdrawn, the more important functions

of the organism come to a standstill—growth and the phenomena of movement as well as the transport of food material (compare Lecture XIV, p. 169) from cell to cell, the movements of protoplasm and of entire organs. We must not forget, too, that oxygen is also an essential food-stuff of the plant, and hence we have for the first time to deal with an *element* as a nutritive material, whilst the nutrients hitherto spoken of were compounds. We are still far from a *complete* understanding of the significance of respiration in the maintenance of vital phenomena, but we can at least claim to possess an approximate conception of it by studying the energy relations of the process. In burning wood or coal energy is liberated which, as every steam-engine demonstrates, is capable of doing work. A transformation of the energy originally present in the material must take place; it must be changed from the potential into the kinetic condition. Similarly, in the physiological combustion of starch or sugar in the plant-cell, kinetic energy is evolved, obviously essential for carrying on the manifold activities of the organism. When organic materials are broken down in the process of intra-molecular respiration energy is also released, although no free oxygen be added, just as when in the breaking down of certain chemical compounds a re-arrangement of their atoms only takes place without the addition of any other element. In the higher plants the energy arising from intra-molecular respiration is insufficient to carry on all the vital phenomena; in our next lecture, however, we shall get to know of organisms where this is so. The heat of combustion of the respiratory materials gives us an approximate idea of the amount of energy released in respiration. If these materials be oxidized down to the final products, water and carbon-dioxide, we thereby obtain bodies which have no heat of combustion, and the whole of the energy is released by respiration. When, however, organic acids or alcohol arise as primary products it is only the difference between the heat of combustion of the materials produced, and the sum of the heats of combustion of the final products that is available for carrying on the work of the plant. In the production of heat which appears to be inseparable from respiration (compare Lecture XXXI) we have the evidence required to show that the chemical energy of the respiratory materials is transformed, still we must remember that the *heat* so produced must obviously be reckoned as lost to the plant. If the production of heat were the chief end of respiration then respiration might be compensated for by heat introduced from without, and we could reduce it by heating the plant; this, however, is quite incorrect, for with every increase in temperature respiration also increases. Under these circumstances it is almost inconceivable that, as RODEWALD thinks (1888), the total energy of the respiratory materials in certain cases appears as heat. One would not expect this to be generally true; one would rather expect, that *in addition to the heat* produced in physiological combustion, *other forms of energy* would appear which might be of service in the plant economy.

We cannot close this lecture without summarizing, if only in a sentence, the history of our position in relation to this subject. The fact that in illuminated green parts respiration is masked by assimilation makes the demonstration of universal respiration extremely difficult. Although SAUSSURE had a clear perception of the fact that respiration was continuous in chlorophyll-bearing regions exposed to light, we should not have been indebted to SACHS (1865) for the first expression of the modern conception of the matter, if LIEBIG had not explicitly denied respiration in plants. The service rendered by SACHS lay essentially in correcting the phraseology previously in use, seeing that GARREAU (1857), had already shown the probability of the existence of respiration in all green parts of plants; before SACHS's time it was customary to speak of a 'day and night respiration', but SACHS, by introducing the terms 'assimilation' and 'respiration' for these two antagonistic processes, did away with many unending misconceptions.

Bibliography to Lecture XVI.

AUBERT. 1892. Revue gén. 4, 203.
[BARNES. 1905. Bot. Gaz. 39, 81.]
BEHRENS. 1901. Centrbl. Bakt. II, 7, 1.
BERNARD, CL. 1878. Leçons s. l. phénom. de la vie. Paris. 1, 278.
BLACKMAN. 1895. Phil. Trans. R. Soc. B, 186, 502.
BONNIER and MANGIN. 1884. Annales Sc. nat. VI, 19, 217.
BONNIER and MANGIN. 1886. Ibid. VII, 3, 5.
BREFELD. 1876. Landw. Jahrb. 5, 327.
CELAKOWSKI. 1892. Flora, 76, 194.
[CHODAT and BACH. 1904. Arch. des Sc. phys. et nat. expér. 17, 477.]
CLAUSEN. 1890. Landw. Jahrb. 19, 893.
DETMER. 1880. Phys. d. Keimung. Jena.
DETMER. 1883. Lehrb. d. Pflanzenphysiologie. Breslau.
[DIAKONOW. 1886. Ber. d. bot. Gesell. 4, 2.]
ELFVING. 1886. Oefversigt Finsk. Vet. Soc. Förh. 28.
EMMERLING. 1903. Centrbl. Bakt. II, 10, 273.
EWART. 1896. Journal Linn. Soc. 31, 408.
GARREAU. 1851. Annales Sc. nat. III, 15, 1.
GERBER. 1900. Congr. internat. de bot. Paris. Compt. rend. p. 55.
HAHN. 1901. Ber. d. chem. Gesell. 34, 3355.
HUNGER. 1901. Ber. d. bot. Gesell. 19, 374.
JACOBI. 1899. Flora, 86, 289.
JOHANNSEN. 1885. Unters. aus d. bot. Inst. Tübingen, 1, 686.
JOHANNSEN. 1896. Bot. Centrbl. 68, 337.
JUMELLE. 1892. Revue gén. 4, 269.
KOLKWITZ. 1899. Jahrb. f. wiss. Bot. 33, 128.
KOLKWITZ. 1901. Ber. d. bot. Gesell. 19, 285 ; Blätter f. Gerstenbau etc. 1901.
KRAUS, G. 1886. Abh. Naturf. Gesell. Halle, 16.
KOSINSKI. 1901. Jahrb. f. wiss. Bot. 37, 137.
KUNSTMANN. 1895. Verh. zwischen Pilzernte u. verbr. Nahrung. Diss. Leipzig.
LASKOWSKY. 1874. Versuchsstationen, 17, 219.
LECHARTIER and BELLAMY. 1874. Compt. rend. 79, 1006.
LIEBIG. 1862. Die Chemie in Anwendung auf Agrikultur u. Physiologie, 7th ed. Braunschweig. 1, p. 29.
[MATRUCHOT and MOLLIARD. 1903. Revue gén. d. Bot. 15, 193.]
MAXIMOW. 1902. Centrbl. Bakt. II, 9, 193.
MAYER, AD. 1879. Versuchsstat. 23, 335.
MAYER, AD. 1875–87. Versuchsstat. 18, 410; 21, 277; 30, 217; 34, 127.
MAZÉ. 1900. Annales Instit. Pasteur, 14, 350.
MOLISCH. 1901. Stud. über Milchsaft etc. Jena. p. 63.
MORKOWIN. 1899. Revue gén. 11, 289.
ONO. 1900. Journal Coll. Sci. Imp. Univ. Tokyo, 13, 1, 141.
PFEFFER. 1885. Unters. aus. d. bot. Instit. Tübingen, 1, 636.
PFEFFER. 1889. Abh. math.-phys. Kl. Kgl. Sächs. Gesell. d. Wiss. 15, 449.
PFEFFER. 1895. Jahrb. f. wiss. Bot. 28, 257.
PFLÜGER. 1875. Archiv f. Physiol. 10, 251.
PURIEWITSCH. 1900. Jahrb. f. wiss. Bot. 35, 573.
RACIBORSKI. 1898. Ber. d. bot. Gesell. 16, 52 and 119. Flora, 85, 362.
RICHARDS. 1896. Annals of Botany, 10, 531.
RISCHAVI. 1876. Versuchsstationen, 19, 321.
RODEWALD. 1888. Jahrb. f. wiss. Bot. 19, 281.
SACHS. 1865. Experimentalphysiologie. Leipzig.
SAUSSURE. 1804. Chem. Unters. über die Vegetation. (German ed. by Wieler, Ostw. Klassiker, 15 and 16. Leipzig. 1890.)
STICH. 1891. Flora, 74, 1.
STOKLASA. 1903. Beitr. z. chem. Phys. u. Path. 3, 473.
VINES. 1901. Annals of Botany, 15, 181.
WARBURG. 1886. Unters. bot. Inst. Tübingen, 2, 53.
WEHMER. 1891. Bot. Ztg. 49; Abstract by Author in Bot. Centrbl. 1894, 57, 104.
WEHMER. 1892. Bot. Centrbl. 51, 337 (Rev.).
WEHMER. 1894. Beitr. z. Kenntn. einh. Pilze, 1.
WEHMER. 1897. Centrbl. Bakt. II, 3, 102.
ZIEGENBEIN. 1893. Jahrb. f. wiss. Bot. 25, 563.

LECTURE XVII

FERMENTATION

THE destructive metabolism which is an essential concomitant of vitality in so far as it consists in the complete combustion of organic materials with the production of carbon-dioxide and water, we have termed *respiration*. In the present lecture we propose to treat of *fermentation*, by which we mean an active metabolism where the oxidation is incomplete or where, instead of oxidation, a decomposition of an entirely different kind takes place. Respiration and fermentation have in common the formation of final products having less heat of combustion and more limited stores of energy than the materials from which they arise. In the formation of these final products energy must therefore be released, and it is this energy which the organism makes use of in some unknown way to carry out its vital activities. As has been shown in the last lecture, respiration and fermentation are not two essentially different processes, for since we found that in the respiration of Fungi, for example, a number of organic acids, such as oxalic, malic, &c., arose as products of incomplete combustion, we are quite entitled to term this process fermentation, and speak of oxalic acid fermentation, malic acid fermentation, and so on, after the chief products produced. The nomenclature of fermentation is not as yet quite consistent since it is sometimes based on the nature of the chief, or to be more accurate, the most noticeable, product—for in alcoholic fermentation carbon-dioxide is as much the chief product as alcohol is—sometimes also after the material that is fermented. Thus by butyric acid fermentation one understands a process in which butyric acid is the most prominent product which arises, but by cellulose-fermentation we mean a process which results in the destruction of cellulose. As an example of another type of fermentation which takes place without the presence of oxygen, may be taken that in which alcohol is formed as a result of intra-molecular respiration. We have already established the fact that this alcoholic fermentation stands partly, at least, in lieu of respiration; so long as intra-molecular respiration continues, the plants we have hitherto been considering cannot develop their full vital capacities—their growth, for example, comes to a standstill—but still they remain alive and regain their ordinary powers after being transferred to normal conditions and after the addition of oxygen. If, however, one places these organisms in an atmosphere free of oxygen—which may be easily done in the case of Fungi—without at the same time permitting the conditions necessary for the formation of alcohol, and if one gives them in place of an easily fermentable sugar another equally nutritive source of carbon, e. g. quinic acid, lactose (DIAKONOW, 1886), they rapidly die. [DIAKONOW'S work has not, however, received complete confirmation, for NABOKICH (1903) and KOSTYTSCHEW (1904) have shown that peptone, quinic acid, and milk-sugar may also support intra-molecular respiration, but nothing like so well as sugar.] Similarly, *oily* seeds cannot respire intra-molecularly so well as starchy seeds (MAZÉ, 1900; GODLEWSKI, 1901) because the alteration of fat into carbohydrate is manifestly impossible without oxygen. There is no ground for distinguishing the process of alcohol formation which takes place in intra-molecular respiration from alcoholic fermentation so called, especially since GODLEWSKI has demonstrated that alcohol and carbon-dioxide occur in the same proportions as in fermentation, and that they also develop in seedlings of Phanerogams from sugar supplied artificially. Still the term 'alcoholic fermentation' always suggests in the first instance a definite organism, viz. *yeast*, because

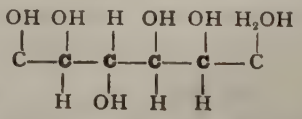
the alcohol formed in the manufacture of wine, beer, and brandy is almost entirely a product of the activity of this organism.

Yeasts belong for the most part to the genus *Saccharomyces*, a genus rich in species and in varieties (Fig. 37), Ascomycetes of the simplest structure increasing by budding. Under certain culture conditions they do not exhibit any capacity for forming alcohol and behave just like other Fungi, respiring organic substance into carbon-dioxide and water. Yeast behaves in this way when grown in a nutritive solution in which peptone serves as a source both of carbon and of nitrogen, or in solutions which in addition to some appropriate source of nitrogen, contain quinic acid or lactose to supply the carbon required. As might be expected the yeast under these conditions dies at once if oxygen be withheld. If the milk-sugar be replaced by cane sugar, alcohol is formed *whether oxygen be present or not.* It is obvious that fermentation begins only when an appropriate fermentable substance is present, so that we must first of all inquire which substances are fermentable and which not. Yeasts can form alcohol only from *carbohydrates*, and at the same time they exhibit a remarkable capacity for distinguishing between bodies nearly related, as we have elsewhere

Fig. 37. Yeast Fungi. a. *Saccharomyces cerevisiae*; b. *S. pasteurianus* III; c. *S. ellipsoideus* I; d. *ellipsoideus* II. (After FISCHER, Vorles. ü. Bacterien. 2nd ed.)

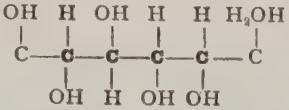
discovered. The proof of this fact, as far as yeasts are concerned, we owe chiefly to E. C. HANSEN (1888) and E. FISCHER (1898), who have established the fact that individual species and varieties behave in entirely different ways.

Fermentable carbohydrates are recognized by possessing three carbon atoms, or a multiple of that number, and are directly fermentable trioses, hexoses and nonoses, whilst the more complicated di-, tri-, and polysaccharides must be first of all hydrolysed and transformed into hexoses before they can be fermented. In nature, only the hexoses and those higher sugars which may be split into hexoses are to be considered as forming the material of fermentation. Amongst these we may distinguish aldohexoses and ketohexoses, the former exhibiting four and the latter three asymmetric carbon atoms. These asymmetric carbon atoms are indicated (in the following formulae) by heavy type, and the first of these we may draw attention to is ordinary grape sugar i.e., *d*-glucose :—

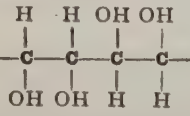
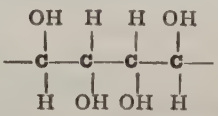


Let us now consider in this formula the four H and OH groups united to the four asymmetric carbon atoms to be arranged in all possible configurations, we shall then have sixteen 'stereoisomeric' hexoses and these are all optically active; eight rotate polarized light to the right and the other eight rotate it to the left. The enantiomorphs, distinguished by their different rotatory powers, are characterized by the interchange of H and OH groups around the asymmetric carbon atoms;

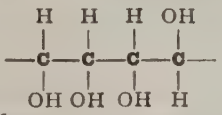
thus, for example, the enantiomorph of *d*-glucose, i. e. *l*-glucose, has the following configuration :—



Many, but not all, of these sixteen different bodies are known, and it has been found that *only* those which rotate polarized light to the right, and even not all of these, are fermentable. Besides *d*-glucose, *d*-mannose and *d*-galactose alone among the hexoses are fermentable, i. e. those whose asymmetric carbon atoms are united to the H and OH groups in the following way :—

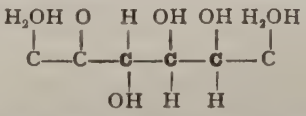
d-mannose  and *d*-galactose 

In contrast to *d*-glucose it is the first carbon atom in *d*-mannose and the third in *d*-galactose which is exchanged, but these exchanges do not prevent fermentation ; if, however, as in *d*-talose, the first as well as the third carbon atoms are exchanged :—



then fermentation is impossible.

Of all the known ketohexoses only one, d-fructose (= laevulose), is fermentable ; its configuration is as follows :—



So far as regards the behaviour of its three carbon atoms it agrees entirely with d-glucose, and hence may be explained the fact that it is nearly as easily fermentable as that sugar ; mannose also closely resembles glucose, and experience shows that it is more easily fermentable than galactose, whose configuration differs more widely. In individual cases we have not as yet learned what factor determines this capacity for undergoing fermentation. Further, all Saccharomycetes do not behave in the same way towards galactose ; *S. pasteurianus* I causes it to ferment almost as quickly as it does the three other hexoses ; *S. ellipsoideus* induces fermentation in it only slowly, *S. productivus* and *S. apiculatus* do not cause it to ferment at all.

The disaccharides are, as already mentioned, not directly fermentable, they must first of all be hydrolysed into hexoses by means of enzymes. Thus ordinary beer and wine yeasts give off enzymes which break down cane sugar very quickly outside the cells into equal parts of dextrose and laevulose. One of these, invertase or saccharase, we have met with before. The products of decomposition are, for the most part, not acted on with equal rapidity; many yeasts consume the dextrose first, some attack the laevulose first. This may depend on a difference in fermentative power, but probably other considerations may play a conspicuous part, e. g. powers of diffusion (KNECHT, 1901). In certain cases, *Monilia candida* for example, we may assume a direct fermentation of cane sugar, for it disappears during fermentation without any invert sugar (= dextrose + laevulose) appearing in its place. Careful research has shown that in this case also an invertase comes into play ; but since it cannot diffuse out of the cell its activity is intra-cellular only.

A large number of yeasts are able to hydrolyse not only cane sugar but maltose also, and to induce fermentation in the resulting products (= two molecules of glucose). Others (*S. marxianus*, *ludwigii*, *exiguus*) attack cane sugar only, others still (*S. apiculatus*, *Schizosaccharomyces octosporus*) maltose only. Maltase must, therefore, be looked on as a distinct enzyme from saccharase. Its recognition was rendered all the more difficult because it was at first to be obtained only from the dead cells by drying, since it was obviously unable to penetrate the living protoplasm. A third disaccharide, lactose (milk-sugar), is broken up by yet other yeasts, which together can also hydrolyse maltose and saccharose. Similarly a fourth natural disaccharide, trehalose, is acted upon by certain other yeasts.

We need not discuss the artificial disaccharides nor the fermentable trisaccharide, raffinose, since they add nothing essentially new to the facts already known. Finally, as to the polysaccharides, e. g. starch, we already know that they also are transformed into sugar by an enzyme, diastase; in general, however, organisms which can produce alcoholic fermentation are unable to use starch, although there are certain Fungi, members of the Mucorineae—though certainly not Saccharomycetes—such as, for example, *Mucor alternans*, *Amylomyces rouxii*, which can do so, and these during the last few years have been employed technically for the purpose.

There is another question which we need only glance at in passing, and that is whether the possession of a definite enzyme and the power of producing fermentation are constant characters of the organism or whether they may be induced to appear in it by culture methods and change of habit. The latter view is held by French investigators, and although, according to the results of the work carried out in the Carlsberg Laboratories, these results do not appear to be entirely reliable (Klöcker, 1901), still it must be said that critical evidence against such a possibility does not exist, since experience has taught us that micro-organisms may be easily affected by culture methods, acquiring or losing certain characteristics according to circumstances.

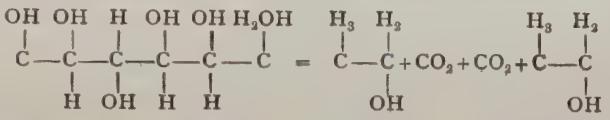
As already noted, sugar is broken down into carbon-dioxide and alcohol during alcoholic fermentation, both of which appear in about equal amounts; thus 100 parts of cane sugar in one of Pasteur's experiments, giving by hydrolysis 105·26 g. of invert sugar, gave rise to 51·0 g. of alcohol and 49·1 g. of carbon-dioxide; the remainder, about 5 g., went to the nourishment of the yeast and the formation of certain secondary products. The proportional relations of carbon-dioxide and sugar is about what one would expect if a molecule of cane sugar, on taking up water, broke down into four molecules of carbon-dioxide and four of alcohol :—

$$\begin{array}{lcl} C_{12}H_{22}O_{11} + H_2O & = & 4\,C_2H_6O + 4\,CO_2 \\ 100\text{ g.} + 5{\cdot}3\text{ g.} & = & 53{\cdot}8\text{ g.} + 51{\cdot}5\text{ g.} \end{array}$$

It is quite possible that the process of fermentation consists in a decomposition of sugar of this nature. As a matter of fact, however, there are always secondary products formed which, in the older analyses at least, must have been due to the presence of foreign organisms in the fermenting fluid; still some of these products, especially glycerine and succinic acid, appear during the action of perfectly pure yeast. The amounts of these substances vary, in the first place, according to the species of yeast employed, and in the second, according to the nature of the food, not only of the fermentable carbohydrate but also of the nitrogenous constituent. Laborde (1899) found that 2·5–7·75 g. of glycerine were formed from 100 g. of fermenting sugar as the result of the action of different yeasts, and Wortmann (1892) also showed that very variable amounts of glycerine were produced by different wine yeasts. Succinic acid comes next to glycerine in amount and reaches about 0·5 g. per 100 g. of sugar; the amount is also liable to considerable variation. Since both these secondary products are formed by yeast under conditions which exclude the formation of

alcohol (UDRANSKI, 1889), we have obviously to do with a special kind of metabolism whose significance is unknown but which has nothing to do with alcoholic fermentation (WORTMANN, 1898). Into the question of the occurrence of other secondary products, such as aldehyde, volatile acids, &c., we need not enter ; these are doubtless formed also in pure yeast fermentations, but they are not actually products of fermentation. There are also other bodies present which give definite characters to the fermented liquor, but these are not due specially to the yeast but to the fermentable material ; in the case of wine they are derived from the grape.

The decomposition of sugar into alcohol and carbon-dioxide is remarkably complete ; this is best seen by a comparison of the structure of *d*-glucose with the products of fermentation :—



It has not hitherto been possible to carry out this decomposition by purely chemical means, and hence it was for long thought that the *living protoplasm* alone had the power to bring it about. Recently, however, E. BUCHNER (1897 onwards) has succeeded, by vigorous friction and under high pressure, in obtaining a sap from yeast, which can induce the formation of alcohol from all the carbohydrates that the yeast acts on. BUCHNER'S view is that fermentation is a purely chemical process, carried out by definite substances present in the expressed sap. BUCHNER'S explanation has not remained unchallenged ; doubts have been expressed in the first place whether the decomposition of the sugar effected by the expressed sap is identical in character with that produced by the living protoplasm, and, secondly, it has been suggested that the activity of the expressed sap is due to living yeast protoplasm contained in it. If that be so, yeast protoplasm must have certainly extraordinary powers of resistance, since after precipitation with alcohol and acetone the redissolved precipitate still retains its activity. Recently, ALBERT (1901) has met these objections by killing the yeast in alcohol and ether, when, notwithstanding this treatment, he found that its power of fermentation was unaffected ; the dead cells were able to form alcohol from sugar, whether in the natural or in the crushed state. The fermentation in the former case is *intra-cellular* as in the case of living cells ; the sugar is split up inside the cells, for the fermenting substance cannot pass through the cell-wall. This, however, is not extraordinary, for it has been established that many enzymes are incapable of passing through the cell-wall. BUCHNER unhesitatingly considers this body as an enzyme and calls it 'zymase'. It must, however, be remarked that zymase has not much in common with the enzymes we have previously studied, for these act by relatively simple methods, e. g. hydrolysis, a power possessed by inorganic agents also. In addition to hydrolysing enzymes we have also oxidizing enzymes to take note of. Zymase differs from all of these in being much more thorough in its action and in possessing the power of breaking down the sugar molecule into new combinations of carbon, hydrogen, and oxygen. [According to BUCHNER'S (1905) observations it would appear that lactic acid is not improbably an intermediate product in alcoholic fermentation.]

But zymase differs from hydrolytic enzymes in another respect. The duration of its activity at summer temperature is very slight ; it is very rapidly destroyed, and in order to obtain large quantities of alcohol much expressed sap and concentrated sugar solutions must be employed, whilst in the case of ordinary enzymes small amounts are able to produce great changes. This distinction may not, however, be of great importance since it is possible that the activity of zymase may be inhibited by the enzymes in the expressed sap ; still the

difference in the activity of enzymes and zymase appears to us to be sufficient to separate these bodies from each other. Since also it is possible that we may find substances associated with other fermentative processes analogous in their action to zymase, we had better employ the name 'zymase' as a collective term for all substances produced by organisms which have the power of inducing fermentative decompositions, and designate BUCHNER'S zymase by the term alcoholase. There can be no doubt that the determination of zymases is one of the greatest advances as yet made in the study of the theory of fermentation; it is therefore worthy of mention that E. TRAUBE (1858) believed 'enzymes' to be the cause of fermentation, although he was unable to separate them from the living substance. The successful *isolation* of alcoholase has as yet not been effected.

Although the existence of alcoholase has been proved it by no means follows that alcoholic fermentation is to be regarded as a vital phenomenon, even although zymases as well as enzymes generally, in their origin and activity, are entirely dependent on organisms. This is all the more apparent when we remember the dependence of fermentation on external conditions. We will confine ourselves to the consideration of two factors only, temperature and oxygen. With regard to temperature we may note that an optimum of 25° C. is necessary for fermentation while the optimum activity of zymase lies much higher. We must go more carefully into the question of the effect of oxygen on fermentation. Doubtless, when zymase operates in a test-tube it is quite immaterial whether oxygen be present or not. From our experience of Fungi and higher plants also, we must expect yeast to produce alcohol only in the absence of oxygen; but in the cases mentioned the formation of alcohol takes place only in intra-molecular respiration and is due to the presence of a zymase; at least, STOKLASA (1903) has found such an enzyme in beet, although GODLEWSKI (1901) was unable to determine its presence in seeds which produced alcohol. [MAZÉ (1904) has shown that STOKLASA'S arguments in favour of general distribution of zymase are not valid.] The *formation* of zymase in beet must come to an end in the absence of oxygen. Yeast, however, behaves quite otherwise; it forms alcohol in presence of oxygen as easily as in its absence. [According to WEHMER (1905) species of *Mucor* behave in the same way.] This fact, obviously of so much importance to the theory of fermentation, deserves more detailed investigation, for it is impossible to believe that alcoholic fermentation is quite independent of oxygen.

Yeast, in addition to its power of inducing fermentation, can also respire in the ordinary way, and if oxygen be present in the fermentable medium part of the sugar will be respired and the rest fermented. A definite quantity of yeast, however, generates all the more alcohol the less normal respiration is permitted, and if oxygen be entirely absent the whole of the sugar disappearing in the course of metabolism will be *fermented*. Since the energy evolved from the respiration of the sugar is much greater than from its fermentation one comprehends that more sugar will be used up in the latter case. In GILTAY and ABERSON'S experiments (1894) 1 g. of yeast (dry weight) transformed 7 g. of sugar in presence of oxygen and 14 g. in its absence in the same time. The maximum formation of alcohol in no way corresponds to the optimum vital conditions of yeast. Growth and increase of yeast are largely dependent on the presence of oxygen; when it is withdrawn vegetative activity ultimately ceases, although fermentation still goes on. There is, unfortunately, no unanimity among the different authorities as to how far increase of the yeast is possible in the absence of oxygen; according to some authors, it ceases entirely, according to others (BEIJERINCK, 1894), twenty to thirty times the original mass may be produced. Whichever view is correct, growth is always limited when alcoholic fermentation only occurs, while it is unlimited when respiration begins. Since, however, the amount of alcohol produced in a unit of time depends obviously on the *amount* of yeast present, one cannot say *a priori* whether a minimum amount of yeast will give in the long run more alcohol in a nutritive fluid con-

taining oxygen or in one in which none is present. Experiments and practical experience have shown that in presence of a *limited* amount of oxygen yeast increases so vigorously and fermentation is so little retarded that the maximum amount of alcohol may be formed. If a very small quantity of yeast be added to a suitable nutritive solution from which air has been fully excluded the organism at first greedily absorbs the oxygen dissolved in the solution; it even makes use of combined oxygen, such as the oxygen united with haemoglobin, although it is unable to decolourize indigo-carmine. As the yeast increases and the alcoholic fermentation begins, bubbles of carbon-dioxide make themselves apparent in the fluid; these, however, become rapidly smaller and finally cease. The addition of a minute quantity of air at once increases the intensity of the fermentation in a remarkable manner, and bubbles of carbon-dioxide once more make their appearance (DUCLAUX, 1900). If oxygen be permanently excluded, the yeast in the end dies, even though food-materials be still present (BEIJERINCK, 1894). *In the long run*, alcoholic fermentation proper is also promoted by the presence of small quantities of oxygen. The statements in the literature on this whole question are, however, more contradictory than in any other department of physiology.

It will perhaps help us to reach an accurate conception of the behaviour of yeasts if we consider here two terms suggested by PASTEUR (1861 and 1863), and generally employed since his time. Such forms as could go through their normal development only in presence of oxygen he termed *aerobic*, and *anaerobic* such as could get on without oxygen, or such as were injured by that gas. In studying typical aerobic plants we saw that a certain partial pressure of oxygen was injurious to them also. This oxygen pressure lies in their case far above that of normal atmospheric air, whilst that affecting typical anaerobes lies far below it. The extremes are connected by transitions which, on the one hand, from their increasing liability to *injury* from oxygen, and on the other in their increasing *need* for that gas, shade quite gradually into each other. Since nothing is known as to the injury done to yeast by oxygen, we can only characterize it by its oxygen requirements. It differs obviously in this point both from typical aerobes and from anaerobes, since it can live and multiply for a long, though not unlimited, time without respiration.

We have not as yet solved the chief question before us, viz. why yeast forms alcohol not merely when its respiration is interfered with but under *all* conditions? From the point of view of energy we have here to deal with a loss, and the question is whether or not gain in another direction does not counterbalance this loss? To WORTMANN (1902) we owe a very probable hypothesis; he considers the alcohol as a protection employed by the yeast against associated micro-organisms, since yeast itself can tolerate 10–18 per cent. of alcohol while all other organisms occurring in saccharine fluids are injured by 4–10 per cent. of alcohol. This theory reminds us of the formation of acids by Fungi, not with the object of gaining energy but for certain biological reasons. The difference between the formation of acids by *Aspergillus* and the formation of alcohol by yeast lies in this, that *Aspergillus* aims at acidifying the substratum, only in order that it may be made unfavourable for *other* organisms; it ceases forming acids before it is itself injured by this product of its own metabolism, while yeast, on the other hand, goes on producing alcohol until it is itself killed by the alcohol, and apparently almost all fermentative actions are similarly brought at last to a standstill by the products of fermentation. This fact does not seem to us to be quite in accord with WORTMANN'S hypothesis. If our definition is to be maintained and we are to speak of fermentation only where destructive metabolism precedes a *gain in energy*, then alcoholic fermentation as well as acid fermentation by Fungi must be excluded from this category of actions. We must also inquire what other fermentative processes exist, since the poisonous effect of the chief product has apparently a definite significance apart from the

gain in energy in these other cases also. We must extend our original definition and say that fermentation is dissimilation which is not carried, as in respiratory combustion, to the final stages. Under these circumstances we must also regard the formation of organic acids in succulents as a case of fermentation.

Attempts to bring other fermentative actions within the scope of our observations land us in a region where the data are most contradictory—more indeed than need be. Up to the time of PASTEUR's first important investigations we knew nothing of what is nowadays known as a 'pure culture'; before that, investigators experimented with cultures usually containing several organisms and they were consequently unable to determine what part each played in the production of the final result; and nowadays, when the need for pure cultures has become generally recognized, the effort to achieve *practical* results in the physiology of fermentation has increased to such an extent that important scientific questions requiring solution have been quite ignored. Hence there has arisen a mass of literature which can only be surveyed by specialists. Under these conditions it is to-day more than ever difficult to give an outline of the present position of our knowledge of this subject. [LAFAR has performed an extremely valuable service in giving in his Handbook of Technological Mycology (Jena, 1904, onwards) an exhaustive account of all the important fermentative processes used in the Arts. This work, which is still in course of publication, may be referred to for further details.]

Alcoholic fermentation, that is to say the formation of ethyl-alcohol, is not confined to yeast; it may be induced also, as we have seen, under certain conditions by Mould-fungi as well as by higher plants, but only by a relatively small number of Bacteria. It is true that certain Bacteria have the power of forming higher alcohols; thus, for example, fusel oils (a mixture of propyl-, butyl-, and especially amyl-alcohols), which are developed during the manufacture of brandy, and particularly of potato spirit, are due apparently to the action of Bacteria; pure yeasts give rise to none of the higher alcohols. BEIJERINCK (1894) has carefully studied a bacterium which induces the formation of propyl- and butyl-alcohols, and to this form we may devote some little attention since it is in many respects an interesting contrast to yeast. *Bacillus butylicus* (*Granulobacter butylicum*, Beij.) (Fig. 38), as it is named, is an elongated rod of considerable size; it contains a large amount of a carbohydrate, the so-called granulose, coloured blue by iodine, and finally forms endospores in more or less spindle-shaped swellings. In nature this bacterium occurs with great constancy on the fruits of certain species of barley and consequently also in the meal formed from them. If such meal be made into a mucilage by cooking for a short time, the spores, which resist, at least for a few minutes, this high temperature, quickly begin to germinate, and the organisms increase rapidly in number. At the same time the starch is changed, by a diastatic enzyme excreted from the cells into maltose, and this later on is in part used in constructing new organisms and in part is fermented. The fermentation consists in the development of hydrogen and carbon-dioxide in varying proportions, while butyl-alcohol is also developed. The net amount of this characteristic product is not very great, amounting as it does only to about 1–3 per cent. of the meal. According to more recent research propyl-alcohol is also formed (BEIJERINCK, 1889; Archiv. néerland. II, 2, 402, note).

Fig. 38. *Bacillus butylicus.* After BEIJERINCK. × 900.

Bacillus butylicus—apart altogether from its specific zymatic capacities—differs, however, from yeast in one noticeable point, namely, in its relation to oxygen; it is strongly anaerobic. In order to obtain butyl-alcoholic fermentation the greatest care must be taken to exclude oxygen from the nutritive substratum, since if sweetwort be used as the culture medium small quantities of oxygen are found to be directly injurious. BEIJERINCK removed most of the

oxygen by pumping it out and by passing hydrogen through it, the remainder he got rid of by adding an easily oxidizable body (sodium hydrosulphide). When free oxygen is completely absent unlimited growth of the bacterium takes place and, at the same time, very active fermentation. But whenever the least traces of oxygen are present the organism begins to assume a somewhat different appearance from the strictly anaerobic form and forms no spores. BEIJERINCK (1894 and 1899) denies that we have here to deal with a vital process which goes on *entirely without oxygen* and without normal respiration, although he succeeded in carrying out seven cultures one after the other without oxygen being present, and although he found an increase from one to many millions taking place—not as in the case of yeast from one to twenty- or thirtyfold only. What led him to this conclusion was specially the behaviour of the bacterium under a cover-glass. Many motile Bacteria strive to reach the concentrations of oxygen which suit them best, and collect in such regions; if they be placed on a slide and covered by a cover-glass only a very limited quantity of oxygen can reach them, for the amount of oxygen dissolved rapidly decreases from the edge of the cover-glass inwards. Genuine aerobes collect at the edge of the preparation, genuine anaerobes, on the other hand, in the centre; *Bacillus butylicus* places itself at a certain distance from the periphery, where a low but not a minimum pressure of oxygen exists. The organism thrives also on other nutritive substrata, e. g. 1 per cent. solution of peptone with ½ per cent. of starch paste, but induces fermentation only when air can enter easily; only in sweetwort can it form many generations one after the other without free oxygen. Hence BEIJERINCK held that the sweetwort contained an oxygen reserve combined in some way and yet accessible to the bacillus, and believed that all Bacteria, even the so-called obligate anaerobes use small quantities of oxygen, and thus that there are no organisms which are able to do without free oxygen. Even if BEIJERINCK be right in his conclusions the distinction between aerobes and anaerobes, or as BEIJERINCK puts it, between aerophiles and micro-aerophiles is still perfectly well marked, for the consumption of oxygen in the latter is still far from being sufficient to render possible a respiration capable of maintaining the necessary supply of energy; fermentation must always assist in the process. It is possible that certain functions are carried out only when respiration takes place, while other functions may be performed at the expense of the energy supplied by fermentation.

The researches of CHUDIAKOW (1896) form a useful supplement to those of BEIJERINCK, and they ought to be mentioned here, although they do not deal with *Bacillus butylicus*, but with two butyric acid-producing Bacteria termed *Clostridium butyricum* and *Bactridium butyricum*. Both these forms are strongly anaerobic; in their vegetative condition they are injured by a brief exposure to ordinary air and are killed by longer exposure; even their spores cannot in the long run resist the action of oxygen, although small quantities of oxygen do not produce any injury. *Bactridium butyricum* can develop quite well in air with a pressure of 5 mm., and *Clostridium* at a pressure of 10 mm., and, what is most noteworthy, the small quantities of oxygen present in the culture are, under these conditions, completely absorbed and utilized by the organism.

CHUDIAKOW'S experiments do not lead us to the conclusion that the development of the butyric acid Bacteria mentioned depends on the presence of combined oxygen in a medium that possesses no free oxygen, in the same way as BEIJERINCK asserted for *Bacillus butylicus*, for they grow on dextrose or saccharose, maltose, starch, lactose, and mannite, in combination with peptone or asparagin, urea, ammonium chloride (but not with nitrates), exactly like anaerobes. Since again, the presence of combined oxygen in wort is (in BEIJERINCK'S researches) problematical, the last word has not as yet been spoken as to the nature of anaerobiosis; yet it is extremely probable that the organisms concerned can live without oxygen. On the other hand, there is always the possibility that

these organisms live during anaerobiosis on *stored* oxygen; at all events, organisms are known which possess such a power (EWART, 1897). There are certain Bacteria which render themselves to a certain extent independent of the presence of oxygen by forming a pigment which has the capacity, even when dissolved out of the cells, of absorbing oxygen, just as the haemoglobin of the blood does, and of combining it in such a way that the oxygen is set free again in any situation not provided with free oxygen, and, in the course of a few hours, diffuses out of the cells. These organisms can, for a long time, maintain normal respiration in a space free from oxygen, at the cost of reserves of oxygen which they have accumulated. How far they make use of this power has not as yet been determined. On the other hand, this power is of great service to anaerobes, whether they are able to live always or only for a certain time without oxygen; for they can live in places where, owing to the activity of aerobes, the oxygen has been used up, and thus nutritive material from which other organisms are debarred will be accessible to them.

Having now learned from a study of the formation of ethyl- and butyl-alcohol, and, earlier, of oxalic and other organic acids, that there are various types of fermentation, which differ very essentially in their oxygen requirements, we may now glance at some of the numberless other types of fermentation, and chiefly at those by which the products of the animal and plant worlds are transformed, through the fermentative agency of lower organisms, into simple bodies which may serve once more as nutrients to the higher plants.

If we begin with the alcohol which arises from the fermentative activity of yeast, we have first of all to notice that this substance is produced artificially not only in brewing, wine-making, and distilling, but that it is generally to be found in nature wherever sugar-containing sap occurs. Thus yeasts or other Fungi always locate themselves on the outer surface of many fruits or in sap expressed from plants by osmotic pressure, and in such situations they are able to induce alcoholic fermentation. Among the chief products so formed there is one, carbon-dioxide, a completely oxidized body, for whose further alteration in the plant an expenditure of energy is necessary (p. 130), and which on that account is of no value to any organism as a material for the support of metabolic changes; the other product, however, alcohol, is relatively poorer in oxygen than sugar, and hence can serve as a source of energy to some types of organisms. An earlier opportunity was taken of noting that alcohol may serve as a source of carbon to many Fungi, and it may be concluded that it may serve to help not only in the construction of the organism but also in respiration. Acetic acid Bacteria (compare HOYER, 1898, HENNEBERG, 1898) oxidize alcohol completely into acetic acid, approximately according to the following formula :—

$$C_2H_6O + O_2 = C_2H_4O_2 + H_2O$$

For this process oxygen in large quantity is obviously necessary. Since carbon-dioxide (in the first instance at all events) is not formed, normal respiration does not take place; only when the alcohol is all decomposed, is acetic acid further decomposed into carbon-dioxide, but doubtfully in the case of all acetic acid Bacteria. At present we cannot say with certainty whether an acidifying of the substratum is the primary object of acetic acid fermentation so as to shut out associated organisms, or whether the object is merely to make use of the chemical energy of alcohol. The latter seems to us to be somewhat improbable because there would appear to be no reason why the formation of acetic acid should cease, and, in support of the former hypothesis, we must remember that the acetic acid Bacteria are more resistant to that acid than other organisms.

Acetic acid Bacteria are furthermore not exclusively confined to alcohol as a medium; they can exist in other substances as well, all of which they are capable of oxidizing. Thus they can transform higher alcohols into fatty acids, e. g. propyl-alcohol into propionic acid, and butyl-alcohol into butyric acid. Some of

them can oxidize glucose into gluconic acid, mannite into laevulose, sorbite into sorbose. In addition, certain acetic acid Bacteria are known to be able to form oxalic acid from sugar and many other organic compounds—though scarcely from alcohols (BANNING, 1902). Sugar is further a good source of carbon for acetic acid Bacteria and can be used for growth purposes along with an appropriate source of nitrogen; many acids also, e.g. acetic acid, serve as food-stuff, while *alcohol* is employed *only* as a *fermentable* substance.

Although acetic acid Bacteria do not make any further use of that acid, another widely distributed organism occurs in nature—known as *Saccharomyces mycoderma*—which does do so, and by the three consecutive activities of yeast, of acetic acid Bacteria and of *Mycoderma*, the sugar is finally transformed into the same end products which arise from it in respiration in the normal plant.

The decompositions described are not the only ones which sugar and related carbohydrates undergo owing to the action of microbes in nature; very often lactic acid or fatty acids arise. Bacteria which produce lactic acid as a *by-product* have been described by the dozen, but only a few form this acid in such quantity that we may speak in this case of lactic acid fermentation. If, as in the case of *Bacillus lactis acidi*, the entire fermentation consists simply in the splitting of one molecule of glucose into two molecules of dextro-lactic acid, or, as in *Bacillus acidificans longissimus*, into two molecules of laevo-lactic acid, there is no question of fermentation in the old sense of the term, since no energy is released in the process and its significance can lie *only* in the exclusion of associated organisms. There are other lactic acid Bacteria, however, which give rise to other fermentation products.

Butyric acid is the only one of the fatty acids which arise during the fermentation process which we need speak of here. Animal anaerobes produce fatty acids also as fermentative products, e. g. propionic acid in *Ascaris* (WEINLAND, 1901, Zeit. f. Biol. 24, 55). Butyric acid may be derived from sugar as well as from lactic acid, and indirectly from the polysaccharides starch, inulin, dextrin, &c.; its development is due for the most part to the influence of anaerobic Bacteria, as to which much more or less accurate information has been accumulated. These forms are very like each other morphologically, and also resemble *Bacillus butylicus* above described. According to BEIJERINCK (1894) the latter is characterized by producing butyl-alcohol only, and never butyric acid, while *Bacillus saccharo-butyricus* (*Granulobacter*, Beij.), which occurs in similar situations to *B. butylicus*, develops from butyric acid, in addition to butyl-alcohol, large quantities of carbon-dioxide and hydrogen. GRIMBERT (cited by DUCLAUX, Traité de Microbiol. vol. IV) has described a bacterium named *Bacillus orthobutylicus*, which, in addition to these bodies, forms acetic acid also, as does a bacterium studied by PERDRIX (cited by DUCLAUX), termed by him 'bacille amylozyme'. In the cases which have been accurately investigated it has been shown that the proportion of fermentation products is by no means constant; thus in the case, e.g., of 'bacille amylozyme', at the beginning the amount of carbon-dioxide is far less than that of hydrogen, though, later on, both occur in about equal quantities; so too at the beginning of fermentation only acetic acid is formed, but not in the later stages. No explanation has as yet been given of the causes of these variations, nor of the appearance of such varied products; we must wait for the future to provide us with an explanation of how the active enzymes are to be found and isolated; especially if it be shown that several different zymases occur in one and the same organism, whose activities are affected in a variety of ways by external conditions.

As we have already said, polysaccharides, such as starch, can be used up by certain butyric acid Bacteria; some also are able to attack cellulose, e. g. the bacterium studied by OMELIANSKI (1902) in WINOGRADSKY'S laboratory, a bacillus of very small diameter (0·2 μ) which forms spherical spores in terminal swellings, but which gives no blue reaction when treated with iodine. It may be

cultivated anaerobiotically in a nutritive solution in which Swedish filter paper forms the source of carbon and an ammonium salt the source of nitrogen, and to which carbonate of lime is added to neutralize the acids which arise. The cellulose becomes at first transparent, then in the course of a few months entirely dissolves and is broken down into acetic acid, butyric acid, traces of other fatty acids, carbon-dioxide, and water. In a definite case, 3·35 g. of cellulose gave rise to 2·240 g. of acetic and butyric acids (in varying proportions), 0·972 g. of carbon-dioxide, and 0·014 g. of hydrogen. Obviously the cellulose is first of all split into simpler carbohydrates ; but it is noteworthy that the bacillus has not as yet been made to develop on any other medium than cellulose.

The cellulose, which is produced annually in enormous quantities by the higher plants, and which once formed is no longer for the most part of any further (metabolic) service to them, is again made available for metabolism, and thus vast quantities of carbon, which otherwise would lie unused, become transformed into humus, turf, and coal, once more to be employed in the support of life. The bacillus mentioned is not the only one which acts in this way. It is frequently stated that methane is also formed from cellulose, and the plentiful occurrence of this gas in places where cellulose is undergoing decomposition is evidence in favour of the correctness of this statement. In a word, OMELIANSKI has succeeded in showing that the cause of this methane-fermentation of cellulose is a bacillus, which appears to be like that described, but thinner and more delicate. It grows in a culture solution like that in which the bacillus producing hydrogen-fermentation thrives, but it ferments the cellulose into acetic acid, butyric acid, carbon-dioxide and methane. OMELIANSKI, in one experiment found that 2·0065 g. of cellulose gave rise to 0·1372 g. of methane, 0·8678 g. of carbon-dioxide and 1·0223 g. of volatile acids. Thus about 50 per cent. of the fermentation products are volatile acids, and among these there is about nine times as much acetic acid as butyric acid.

The Bacteria causing hydrogen and methane-fermentation occur frequently in conjunction in nature and it is extremely difficult to separate them. As long as this separation is not effected the products of the fermentation set up by *both* organisms appear in the culture, sometimes those of the one predominating, sometimes those of the other. We may suppose, therefore, that the contradictory results arrived at in other fermentation experiments are often due to impure cultures, and hence OMELIANSKI'S work is of extreme value from the point of view of technique. [OMELIANSKI, 1904 a.]

In addition to cellulose, pectins are among the substances which go to the construction of the cell-membrane. They also are not dissolved out by the plant, but remain in the fallen leaves, twigs, &c., and are attacked by definite micro-organisms in the soil or in water. We have to thank WINOGRADSKY (1895) and BEHRENS (1902) for proof that certain Bacteria, apparently butyric acid Bacteria, carry out 'pectin fermentation' in nature, though we are not as yet accurately acquainted with the products of fermentation. Many species of *Mucor* also are able to dissolve pectins. This dissolution of pectin compounds plays a part also in the technique of hemp and flax manufacture ('retting'), since the isolation of the fibres of these plants is possible only after the dissolution of the middle lamella by pectin-fermentation. [OMELIANSKI, 1904 b.]

On studying the final products of fermentation, e. g. cellulose-fermentation, we find that they are partly fully oxidized products (carbon-dioxide), partly products of extreme reduction (methane, hydrogen), partly intermediate substances (acetic acid, butyric acid, &c.). All the compounds which are incompletely oxidized may still be made use of. Although the actual employment of such energy as may be obtained by oxidation of methane or hydrogen is not as yet known, substances like lactic, butyric, and many other organic acids are, on the other hand, used for respiratory or fermentative purposes by numerous micro-organisms. [The most thoroughly studied of these organic acid fermentations

is that of formic acid (Omelianski, 1903).] The numberless bodies which arise in consequence of these decompositions need not be referred to at present; nor need we treat of the various fermentation products of the higher alcohols, e. g. glycerine, mannite, dulcite, &c. We need only note that none of the products of such fermentations accumulate in nature, but that other organisms are always at hand which have the power of breaking down these primary products, till finally the organic substances are transformed into simple inorganic compounds which are once more available for the nourishment of *higher* plants. Thus we begin to appreciate the fact that over the earth's surface there exists a perpetual transformation, a continuous circulation of materials which we will take a later opportunity of once more referring to (Lecture XIX).

But it is not only the simple organic bodies, such as carbohydrates, acids, and alcohols, which are broken down by the fermentative power of micro-organisms, when these bodies are removed from the living organism, but complicated bodies, and indeed the most complicated of all, the proteids, undergo the same fate. In the first place, these proteids are broken down more or less completely by various excreted enzymes, and the products so formed suffer appropriate fermentations. Although Bacteria are known which are able to produce fermentation in proteids, and although much research has been carried out on the resulting products, we are in no case so fully acquainted with the chemical and biological conditions of such fermentations that we can present a complete picture of the course of the fermentative process. Our remarks on the subject must therefore be brief. When proteid is acted on by anaerobic Bacteria, we term it *putrefaction*, and this is characterized by the appearance of evil-smelling compounds (indol, skatol, &c.), but in the presence of air these substances disappear. In nature these processes go on hand-in-hand as, for example, in the putrefying bodies of animals or plants; soon the aerobic forms have consumed all the oxygen in the interior of the body, and the anaerobic forms then proceed to carry out a further decomposition which we term putrefaction. Owing to the activities of a series of living organisms following each other or living side by side, the proteid molecules, after passing through numerous intermediate stages, are finally broken down into a few simple bodies, viz. carbon-dioxide, methane, hydrogen, ammonia, nitrogen, sulphuretted hydrogen and phosphoric acid.

In the following lectures we will return to the consideration of certain of these final products of proteid fermentation.

Bibliography to Lecture XVII.

Albert. 1901. Centrbl. Bakt. II, 7, 473.
Banning. 1902. Centrbl. Bakt. II, 8, 395.
Behrens. 1902. Centrbl. Bakt. II, 8, 114.
Beijerinck. 1894. Archives néerlandaises, 29, 1.
Beijerinck. 1899. Ibid. II, 2; Centrbl. Bakt. 1900, II, 6, p. 341.
Buchner, E. 1897. Alkoholische Gärung ohne Hefezellen (Ber. d. chem. Gesell. 1897, p. 117; also numerous papers in the same Journal, from 1897 onwards. Compare also the summaries in Bot. Ztg. 1898 onwards).
Buchner, E. and H. and Rapp. 1903. Die Zymasegärung. Munich and Berlin.
[Buchner and Meisenheimer, 1905. Ber. chem. Gesell. 38, 620.]
Chudiakow. 1896. Zur Lehre von d. Anaerobiose. Review by Rothert, Centrbl. Bakt. 1898, II, 4, 389.
Diakonow. 1886. Ber. d. bot. Gesell. 4, 2.
Duclaux. 1900. Traité de microbiologie, 3, 308. Paris.
Ewart. 1897. Journal Linn. Soc. Bot. 33, 123.
Fischer, E. 1898. Zeit. f. physiol. Ch. 26, 60–87.
Giltay and Aberson. 1894. Jahrb. f. wiss. Bot. 26, 543.
Godlewski and Polzeniusz. 1901. Bullet. Acad. de Cracovie.
Hansen. 1888. Rech. s. la phys. et la morphologie des ferments alcooliques, VII. Action des ferments alcool. sur les diverses espèces de sucre (Meddel. f. Carlsberg Laborat. 2, Heft 5).

HENNEBERG. 1898. Koch's Jahresbericht, 9, 249 and 251.
HOYER. 1898. Rev. in Koch's Jahresbericht, 9, 242.
KNECHT. 1901. Centrbl. Bakt. II, 7, 165.
KLÖCKER. 1901. Centrbl. Bakt. II, 7, 152.
[KOSTYTSCHEW. 1904. Jahrb. f. wiss. Bot. 40, 563.]
LABORDE. 1899. Compt. rend. Paris, 129, 344. (Koch's Jahresbericht, p. 98.)
MAZÉ. 1900. Annales Instit. Pasteur, 14, 350.
[MAZÉ. 1904. Annales Instit. Pasteur, 18, 535.]
[NABOKICH. 1903. Ber. d. bot. Gesell. 21, 467.]
OMELIANSKI. 1902. Centrbl. Bakt. II, 8, 193.
[OMELIANSKI. 1903. Ibid. II, 11, 177.]
[OMELIANSKI. 1904 a. Ibid. II, 11, 369.]
[OMELIANSKI. 1904 b. Ibid. II, 12, 33.]
PASTEUR. 1859. Annales de chim. et de phys. III, 58, 347.
PASTEUR. 1861. Compt. rend. 52, 344; 1863, 56, 416 and 734.
STOKLASA, JELINEK and VITEK. 1903. Beitr. z. chem. Phys. u. Path. 3, 460.
TRAUBE, E. 1858. Theorie der Fermentwirkungen. Berlin.
UDRANSZKI. 1889. Zeit. f. phys. Chem. 13, 539.
WEHMER. 1905. Centrbl. Bakt. II, 14, 556.
WINOGRADSKY. 1895. Compt. rend. Paris, 121, 742.
WORTMANN. 1892. Landw. Jahrb. 21, 901.
WORTMANN. 1898. Ibid. 27, 631.
WORTMANN. 1902. Weinbau u. Weinhandel.

LECTURE XVIII

SULPHUR AND NITROGEN BACTERIA

AT the conclusion of our last lecture we noted that the sulphur of the proteid molecule was set free as sulphuretted hydrogen in the course of putrefaction. There are, however, other processes in nature in which sulphuretted hydrogen is also developed. In many of these also micro-organisms are concerned, such as in the reduction of sulphates, which is carried out with tolerable completeness not only by the strictly anaerobic *Spirillum desulphuricans* (BEIJERINCK, 1895), but also by other less exclusively anaerobic forms (BEIJERINCK, 1900). We are unable, however, to explain what service such reductions are to these Bacteria. [According to DELDEN (1903) it consists in the gain of oxygen from the sulphates, just as, in the other reduction processes (p. 232), oxygen is obtained from compounds containing it.] In any case it is not our purpose to enter into a detailed discussion of these processes; what we are interested in at the moment is merely the final product, especially the sulphuretted hydrogen, and the further alterations it undergoes under the influence of certain Bacteria, which for that reason have received the name of 'sulphur-bacteria'.

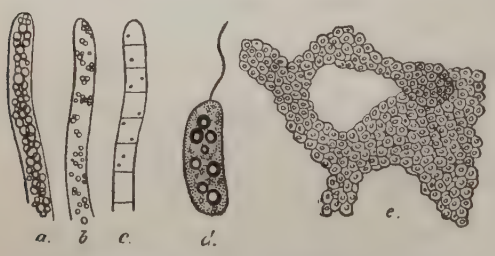


Fig. 39. Sulphur-bacteria, *a–c, Beggiatoa*, × 1000. *d, Chromatium okenii*, × 900. *e, Lamprocystis roseo-persicina*, × 500. After FISCHER, Vorl. üb. Bakt. 2nd edit.

We may select as our first example of sulphur-bacteria, the genus *Beggiatoa* (Fig. 39, *a*), which may be described briefly as a colourless *Oscillaria*, in whose protoplasm plentiful aggregations of sulphur particles or drops may be found. The presence of such large quantities of pure sulphur in its cells leads us to conclude that the sulphur plays an important part in the life of the

organism, and detailed research confirms this view. For long *Beggiatoa* was believed to be the cause of the *formation* of sulphuretted hydrogen, until HOPPE-SEYLER (1886) proved that this was not the case, but that *Beggiatoa* gave rise to sulphur by *oxidizing sulphuretted hydrogen* ($H_2S + O = H_2O + S$). More recently WINOGRADSKY (1887), in a classical monograph, traced out the process in an exhaustive manner, and showed what its significance was in the economy of the organism. [OMELIANSKI (1904) has also provided us with a comprehensive exposition of the physiology of the sulphur-Bacteria.]

Beggiatoa occurs in nature in the mud associated with salt or fresh water, wherever the water or mud contains a sufficient quantity of sulphates. The part these sulphates play, however, is only to supply the material from which other organisms may develop sulphuretted hydrogen. When sulphuretted hydrogen is itself present in the water sulphates are quite superfluous. *Beggiatoa* then appears in the sulphur-containing medium, and develops in it luxuriantly. WINOGRADSKY proved that *Beggiatoa* was present in ever decreasing numbers when the source of sulphur was gradually diminished, and that when the sulphuretted hydrogen was completely absent from the water, *Beggiatoa* also disappeared.

These observations of natural conditions teach us how important sulphuretted hydrogen is for the maintenance of the life of *Beggiatoa*, but convincing evidence and a more exact knowledge of this phenomenon are to be obtained by cultures. If we attempt to cultivate *Beggiatoa* by the methods employed for the majority of Fungi and Bacteria, presenting it with a solid or fluid substratum rich in organic material, it dies off in a very short time. If, on the other hand, we place a small quantity of *Beggiatoa* on a slide, cover it, and keep supplying it daily with fresh quantities of water containing sulphuretted hydrogen (WINOGRADSKY used natural water obtained from the Langenbrücken Baths, to which more sulphuretted hydrogen was added), it not only remains alive, but increases so rapidly that large quantities have to be removed in order to make room for the development of what is left. With the aid of such a micro-culture in a healthy state of growth we are able to carry out easily the following decisive experiments :—

1. The culture is treated twice daily with Langenbrücken sulphur-water, which has been deprived of its sulphuretted hydrogen by being exposed to air. The *Beggiatoa* soon loses its sulphur, and is unable to form more ; it then gradually dies off.

2. If the culture, on the other hand, be supplied with the same water containing sulphuretted hydrogen, *Beggiatoa* again develops rapidly.

The only difference between the two cultures is the absence in one of them of sulphuretted hydrogen, and hence it follows that this substance is essential to *Beggiatoa*, and that from it *Beggiatoa* manufactures the sulphur found among the cell contents. Since this is possible only by oxidation, *Beggiatoa* is absolutely dependent on the presence of oxygen, although it requires this element in quite definite quantities ; too little of it is as disadvantageous as too much. If the experimenter desires to regulate the amount of oxygen supplied to the culture he will meet with insurmountable difficulties, which at once disappear if the organism itself be allowed to regulate its own supply of oxygen. Since *Beggiatoa* is a free motile form, it is able, just like other motile organisms (p. 215) to *find for itself* the optimum concentration of oxygen, provided all possible variations in oxygen tension occur between the edge of the cover-glass and the centre. If we allow a drop of dilute sulphuretted hydrogen-water, free from *Beggiatoa*, covered with a cover-glass to stand in a moist chamber, we see after a few hours that the formation of particles of sulphur which takes place under the influence of air, is apparent only for a distance of a millimetre from the edge of the cover-glass, while the central region remains for long unoxidized, provided that, by fre-

quent renewal of the fluid, we maintain a constant proportion of sulphuretted hydrogen. If we now introduce under the cover-glass some active plants of *Beggiatoa*, we can see the filaments rapidly wandering towards the edge of the preparation, and forming a thick white border (visible to the naked eye) at a distance of about 1 mm. from the edge of the cover-glass. *Beggiatoa* avoids the periphery of the drop, where oxygen is abundant, and also the central region, which contains no oxygen. On the fluid being renewed, however, the filaments retreat more and more to the centre of the preparation as the sulphuretted hydrogen gets gradually used up. When a *Beggiatoa* filament has found the region of optimum oxygen tension, it is able by small changes of position to reach zones either where the sulphuretted hydrogen is abundantly present, or where oxidation of that gas can take place.

The behaviour of *Beggiatoa* in nature conforms to that which it exhibits in a microscopic culture. In a mud swamp, just as in the culture, it always attempts to find a region with an optimum oxygen tension, as it lives on the surface of the culture, inhabiting places subject to overflow and avoiding deeper hollows. Hence both oxygen and sulphuretted hydrogen play a part in determining its distribution, since a definite and not excessive concentration of that substance is essential to its existence.

But *Beggiatoa* not only accumulates sulphur in its cells, but also dissolves it out, and both processes may go on simultaneously, though it is not possible to note this fact directly. The removal of sulphur from the cells can only be determined if formation of the substance is prevented by withdrawal of sulphuretted hydrogen. The amount of sulphur removed in this way is enormous. When WINOGRADSKY supplied an active culture every two or three hours during one day with fresh supplies of Langenbrücken water containing sulphuretted hydrogen, the filaments became full of sulphur by evening (Fig. 39, *a*), and after the supply of sulphuretted hydrogen had ceased, the whole of the sulphur accumulated became dissolved out in twelve to fifteen hours. Fig. 39, *b*, shows the filaments after remaining for twenty-four hours without any sulphuretted hydrogen, while Fig. 39, *c*, represents the same culture after an interval of forty-eight hours. According to WINOGRADSKY'S estimates, the protoplasm of one cell uses up daily an amount of sulphur equal to four or more times its own weight. Such quantities as these render it impossible that the sulphur is devoted to the formation of proteid or the synthesis of any other substances, since *Beggiatoa* grows relatively slowly, only rarely doubling the length of its filaments in twenty-four hours. As a matter of fact, it can be shown that the sulphur subserves an entirely different purpose; it is oxidized in the cell, and the sulphuric acid so formed in turn attacks the carbonates taken into the cell from the water, and passes back again to the medium in the form of calcium sulphate. *Beggiatoa* oxidizes the sulphuretted hydrogen into sulphuric acid, and to some extent accumulates the intermediate product, sulphur, as a reserve. If the supply of sulphuretted hydrogen be limited by offering to the plant extremely dilute solutions, it is possible to grow these Bacteria without their showing any accumulation of sulphur in their cells. Just as the formation of sulphur from sulphuretted hydrogen may be diminished, so also may the formation of sulphuric acid from sulphur, without the aid of the Bacteria; while, however, the former process appears to go on in the cells of *Beggiatoa* about as actively as in water, the organism has obviously the means of greatly accelerating the formation of sulphuric acid (possibly by means of an enzyme ?).

The oxidizing process above described, which, as we have said is absolutely essential to the existence of *Beggiatoa*, is one which is eminently characteristic of this organism, but which is absent in the vast majority of cases. Yet it is not the only peculiarity of this remarkable plant. It possesses neither chlorophyll nor any other allied colouring matter which would lead us to imagine that it was

autotrophic; it has always been considered as a heterotrophic form. In WINOGRADSKY'S micro-cultures in which vigorous increase took place, the Langenbrücken mineral water was the only nutrient supplied, and that fluid contains nitrogen only as traces of ammonia and nitric acid, and only 0·0005 per cent. of organic materials, i. e. in infinitesimal quantity. These are sufficient, as we have seen, to support both life and growth, although they are qualitatively not of a kind one would reckon as of nutritive value. According to FRESENIUS'S investigations, the nutrients must, in part at least, consist of formic and propionic acids. When WINOGRADSKY employed solutions containing sugar, peptone, asparagin, &c., he was unable to obtain as good cultures of *Beggiatoa* as in Langenbrücken water, indeed, for the most part, the plants rapidly succumbed to such treatment.

The general conclusion which WINOGRADSKY arrived at, as based on his experiments on *Beggiatoa*, is as follows:—The oxidation of sulphuretted hydrogen to sulphuric acid is a process in the course of which energy is set free, and in *Beggiatoa* this energy takes the place of that released normally in respiration. While ordinary organisms must devote organic substance, or even part of their own bodies to respiratory purposes, *Beggiatoa* respires sulphuretted hydrogen, and thus saves its organic constituents. It is thus conceivable that it makes such moderate demands on nutrients, both in quality and quantity, that it employs these only for constructing its body and not as a means of supporting vital processes (p. 229). WINOGRADSKY does not deny the possibility of the existence of a normal respiration taking place in addition to the oxidation of sulphuretted hydrogen, but he does not consider it probable.

To *Beggiatoa* are related on the one hand the colourless sulphur-bacteria (species of *Thiothrix*), which are precisely like it in all essentials (though WILLE, 1902, Biolog. Centrbl. 22, 257, has recently denied the occurrence of sulphur in *Thiothrix*; compare MOLISCH, 1903, Bot. Ztg. 61, 57), on the other hand, there is a large number of so-called red sulphur-bacteria (Fig. 39, *d*, *e*) which physiological research has shown to exhibit important differences from *Beggiatoa*, although no satisfactory conclusions have as yet been reached with regard to them. They are distinguished in the first place by possessing a red colouring matter (bacterio-purpurin) of varying tint, but its characters so far as they are known afford no indication of its physiological significance. These Bacteria are further distinguished from *Beggiatoa* by their mode of occurrence, since they prefer water containing large amounts of sulphuretted hydrogen, and are not injured even when that compound is present in a concentrated condition. They are, apparently at least, anaerobic, and avoid situations where oxygen is abundant. Finally, they prefer *light* to darkness and move towards it, whilst *Beggiatoa* avoids light. In common with the latter they make use of sulphuretted hydrogen. Since they live in concentrated solutions of this gas, the question as to how they are able to obtain the necessary oxygen must not be overlooked. According to WINOGRADSKY (1888, b), they always live in association with other micro-organisms which are provided with chlorophyll, and which therefore can decompose carbon-dioxide and give off oxygen. The red sulphur-bacteria then absorb the traces of oxygen present, and use it for oxidizing sulphuretted hydrogen. WINOGRADSKY, in fact, was able to cultivate red sulphur-bacteria only when he associated them with green forms of this kind.

ENGELMANN (1888) offers another explanation of this phenomenon. He has proved by means of the bacterium method previously described (p. 105) that the red sulphur-bacteria decompose carbon-dioxide with the aid of light, and especially of the ultra-red rays, and employ the oxygen so obtained in oxidizing sulphuretted hydrogen. This view of the phenomenon may be preferable, inasmuch as it gives a special function to the presence of bacterio-

purpurin, making it a substitute for chlorophyll. Further, the need for sunlight in the assimilatory activity of the sulphur-bacteria is thus explained, while according to WINOGRADSKY's view, its significance is only indirect. It is to be noted, however, that ENGELMANN's view is by no means established, and that WINOGRADSKY's criticisms (1888) still remain unanswered. It is to be hoped that these interesting problems may soon receive a final investigation and explanation.

WINOGRADSKY believed that another biological group of Bacteria should be associated with the sulphur-bacteria, to which he gave the general name of *iron-bacteria*. They are able to turn iron protoxide into iron oxide, and to benefit by means of this oxidation just as the sulphur-bacteria do from the oxidation of sulphuretted hydrogen. Unfortunately WINOGRADSKY has not followed up his short preliminary note by a detailed treatise, and meanwhile MOLISCH has failed to confirm his results; hence at present we can present no definite data on the subject of iron-bacteria. [Compare also RULLMANN, 1904.] For our present purpose, however, this is of little consequence, since iron-bacteria play by no means so important a part in nature as do the sulphur-bacteria. These latter work for the benefit of the higher plants, inasmuch as they enable the sulphuretted hydrogen formed in the process of putrefaction or otherwise to become once more available for the nutrition of the green plant.

We saw at the conclusion of the preceding lecture, that in addition to sulphur, another even more important element was similarly transformed in the process of putrefaction into a condition in which it was of no service to the higher plant. We found that free nitrogen and ammonia were among the final products of putrefaction, and that the nitrogen was *never* used by the green plant, and the ammonia *much less frequently* than nitrate. We have yet to answer the question whether these materials, like sulphuretted hydrogen, undergo alteration by the activity of definite micro-organisms, so that they become once more available as food, and we will first deal with the question as far as regards ammonia. We may note, to begin with, that all the ammonia does not arise from the fermentation of proteid, but that there are many other important sources of this substance.

Only rarely are katastates containing nitrogen formed in the plant which are unable to undergo further elaboration (compare p. 200, the formation of ammonia by Hyphomycetes); on the other hand, animals regularly give off nitrogen, especially in urine, which contains it in abundance in the form of urea, uric acid, and hippuric acid. It has long been known that these substances form unsuitable sources of nitrogen for autotrophic plants, and it is all the more important to know that they may undergo alteration in the soil into nutrients that are of value. In these processes micro-organisms also play a great part. The best known is the alteration of urea into carbonate of ammonia, a process which has often been termed 'urea fermentation'. This takes place according to the formula :—

$$CO(NH_2)_2 + 2H_2O = CO_3(NH_4)_2$$

the process being thus a simple case of hydration, such as accompanies the action of many enzymes, but without any splitting. If we limit our conception of fermentation to such processes as result in a gain of energy, then the formation of carbonate of ammonia must be excluded from fermentative actions. If, however, we consider the hypothesis referred to at p. 213, we may speak here also of fermentation, since in all probability the significance of the process in the organism is biological only. We can at least suggest that the markedly alkaline reaction acts in the same way as acids and alcohol do (i. e. as a poison) in preventing the presence of other concurrent organisms, since it is a fact

that many organisms are injured by carbonate of ammonia, even in minute doses. At all events, any further elaboration of the ammonia is unknown and improbable, more especially it must not be assumed that ammonia arises from the urea in the same way as it does from a pure peptone nutrient. Since urea does not act as a source of carbon to uro-bacteria they cannot thrive on it alone (BEIJERINCK, 1901); it supplies the nitrogen need only. As far as their carbon requirements are concerned the individual forms behave very differently; species which get on in acetic or oxalic acid are least exacting, but these form only small quantities of carbonate of ammonia; those which grow in tartaric acid produce larger quantities, and those which live in malic acid still more. The greatest amount of ammonia is formed by *Urobacillus pasteurii* and *Urococcus ureae*, which use bouillon as a source of carbon, for in a thin sowing they completely transform 10–12 g. of urea in 100 g. of fluid in the course of a few days. The immediate factor in the formation of ammonia is an enzyme, urease, as to whose occurrence or absence many contradictory statements have been made. As the result of BEIJERINCK'S recent work, its existence can no longer be doubted; for this author was able to prove that uro-bacteria killed by the action of chloroform acted as effectively on urea as living forms. He also established the fact that the urease cannot diffuse out of the cells, so that previous statements as to the solubility of urease are to be explained by assuming that some minute Bacteria in the fluid had been overlooked. [MIQUEL (1904) holds that the view taken as to the solubility of urease is correct.] Ammonia also arises from uric acid; we need not trace the fate of hippuric acid at present.

Ammonia, in whatever way it arises, is, as we have already said, absorbed and retained in the soil, and undergoes, as we shall see, change into nitrous and nitric acids. This process, known as *nitrification*, taking place everywhere in arable soil, was previously considered as a simple oxidation due to inorganic agencies. The observations of SCHLÖSSING and MUNTZ (1877–1879) on the dependence of nitrification on external conditions, and especially the effect of temperature and anaesthetics on them, could only be explained by assuming the action of lower organisms. From such a conception of the process to the certain isolation of the operative Bacteria was a further and more difficult step. Several authors attempted, with the aid of the usual bacteriological methods (nutrient gelatine), to isolate the nitro-bacteria from the soil, and not infrequently they succeeded in obtaining pure cultures, to which they ascribed the power of inducing nitrification. But nitrification took place within such circumscribed limits that it was impossible to avoid the suspicion that the nitrate reaction obtained from the cultures was due less to the activity of Bacteria than to the absorption of nitrates from the air. Nitrates occur abundantly in the air, especially of laboratories, and it is well known that nitrates are vigorously absorbed by alkaline fluids (BAUMANN, 1888).

The labours of WARINGTON (1888) and FRANKLAND (1889) have resulted in an advance in our knowledge of this subject; but it is due to WINOGRADSKY (1890, 1891) that the physiology of the nitro-bacteria has been ultimately cleared up, and his work has the right to be considered as one of the most important discoveries in physiology. It is essentially WINOGRADSKY'S description that we shall follow in our account of the phenomenon. [WINOGRADSKY, 1904.]

His experiences in dealing with the sulphur- and iron-bacteria had trained this investigator perfectly for the study of the nitro-bacteria. He had in those cases to deal with highly characteristic types of organism, distinguished from the mass of Bacteria by their varying behaviour to organic food materials. The methods of isolation and culture of technical bacteriology which he had employed in his experiments had completely failed; might not these failures in the case of the nitro-bacteria be explained by the fact that these organisms require

special conditions of investigation, and cannot be treated in accordance with ordinary methods ?

WINOGRADSKY started from the idea that nitro-bacteria, like sulphur-bacteria, were injured by good organic nutrients, and therefore he attempted to cultivate them first of all in a solution which, in addition to the necessary minerals contained only potassium tartrate to serve as the source of carbon, and ammonium chloride as the source of nitrogen and as nitrification material. When small traces of natural soil, in which nitrification was known to occur, were added to this fluid the results desired were not forthcoming, even although all sorts of changes were made in the concentration of the nutrient fluid. Since, however, the observations of HERAEUS (1886) had proved that organic bodies had little effect, the nutritive solution previously used was prepared *without potassium tartrate.* The result was in the highest degree remarkable ; a vigorous nitrification at once began in the fluid, and hence the line of further research was clearly indicated. After it had been shown that a carbonate of an alkaline earth had a favourable influence, the following nutritive solution was always used :—

Water (Zürich Lake)	1000 g.
Ammonium sulphate	1 g.
Potassium phosphate	1 g.
Basic magnesium carbonate	5–10 g.

If 100 cc. of this solution after sterilization be impregnated with the smallest possible drop of an old culture of a similar kind, a strong potassium nitrate reaction may be obtained in the course of a few days, and in a fortnight all the ammonia contained in the flask is found to have been transformed, while scarcely any change can be distinguished in uninoculated control solutions. In such a culture very many types of Bacteria and other micro-organisms occur, whose number is reduced by repeated transference to similarly constituted nutritive solutions, but finally it was found no longer possible to reduce in this way the number of types present. Five organisms were still found in the thin scum which was obvious on the surface of the fluid, and among these WINOGRADSKY sought for the assumed organism which excited nitrification and absorbed oxygen greedily. Each of these five organisms was investigated separately, but none of them were found to be the cause of nitrification. The real agent was finally discovered in another part of the culture, viz. on the sediment formed from the magnesium carbonate, in the form of a bacterial zoogloea derived from motile oval Bacteria, which for a short time at the beginning swarmed in the fluid. After the significance of this bacterium was established, WINOGRADSKY had still the greatest difficulty in obtaining a pure culture of it. Although his studies are extraordinarily rich in lessons on methods, we will not enter into them further at present, but rather confine our attention to the results he obtained with the use of pure cultures, and study first of all nitrification itself, and then the peculiar behaviour of the bacterium in relation to cultivation with carbon.

In the course of his studies, WINOGRADSKY soon noted that nitrification was actively advanced if ammonia was added only in small quantity, and at once replaced when used up. Consequently, he invariably added to his cultures only 0·04–0·1 g. of ammonium sulphate at one time, and he was able to obtain a very observable nitrification after the second addition. One culture, for example, oxidized 860 mg. of ammonium sulphate in thirty-seven days, another 930 mg. in thirty days, i.e. on an average 4·93 to 6·6 mg. of nitrogen were nitrified per day. What was surprising in these cultures was that the whole of the nitrogen was not oxidized into nitrate, but that a part, *variable* in quantity, was always changed into nitrite. G. and P. FRANKLAND (1890) also observed the formation of *nitrite* in their cultures, while in the soil all the ammonia is altered into *nitrate.* WINOGRADSKY was at first disposed to regard the formation of nitrite as

a result of unfavourable culture conditions, and endeavoured to aid the access of air by employing larger and more extensive layers of fluid. He obtained indeed a very marked increase in nitrification (to as much as 22·7 mg. of nitrogen per day) on spreading a culture, which had previously oxidized 9 mg. of nitrogen, over a surface four times as great in extent. But the effect he expected did not take place, for instead of a *decrease* he obtained an *increase* in the amount of nitrite. The cause of the formation of nitrate and nitrite obviously lay deeper, and WINOGRADSKY was soon able to prove that the formation of nitrite takes place first, and that only after all the ammonia is used up a further oxidation of the nitrite to nitrate follows.

The next question to be answered was, did one and the same organism carry out the formation both of the nitrite and of the nitrate—very much as, according to many authors, the acetic acid-bacteria first turn alcohol into acetic acid, and then respire the acetic acid—or was there a distinct organism concerned in each of the two stages, one forming nitrite, the other nitrate ?

There were certain indications that in all probability the latter view might be the correct one, because when a new culture was taken from one in the stage of most active nitrite formation, *only nitrite* formation went on in it. It was certainly possible that the organism had become altered, and had lost its power of forming nitrate, but it appeared more probable that by a lucky accident only the nitrite organism had been transferred to the new culture, and that in the previous experiments at least two organisms were operating one after the other, by whose combined action the transformation of ammonia into nitrate was effected. In his experiments WINOGRADSKY had now advanced conclusive evidence of the existence of two kinds of nitro-bacteria, one of which constructed nitrites the other nitrates only. Both kept to their specific functions, the one operating on ammonia, the other on nitrite ; other kinds of nitrogenous bodies, such as urea, asparagin, proteid, &c. (OMELIANSKI, 1899), cannot be nitrified at all, nor can the nitrate organism oxidize either phosphorous or sulphurous acids (OMELIANSKI, 1902).

Fig. 40. Nitro-bacteria : *a*, from Zürich; *b*, from Java; *c*, from Quito. × 1000. After FISCHER (Vorles. ü. Bakt. 2nd ed.).

Only a few words need be said as to the morphology of these organisms (WINOGRADSKY, 1892). The nitrite organism is apparently similar in appearance in all European countries, viz. an oval, at some period motile, bacterium (Fig. 40, *a*). Those obtained from Java and other non-European countries resemble the European type (Fig. 40, *b*), but in America other forms, related to the coccus have been found. The nitrate-bacteria, so far as is known, are short rods (Fig. 40, *c*).

The nitrite- and nitrate-bacteria, as we have seen, behave very differently in relation to the nitrogenous compounds which they can oxidize, but quite similarly in relation to carbon. Both in fact not only *require* no organic carbon, but are actually injured by it. Whence then do they obtain their carbon? That is the important question we have yet to answer. In his first experiments, in which simple mineral solutions without any organic additions were used, WINOGRADSKY took special care that all the materials used, the culture and vessels and fluids, were absolutely free from organic impurities, and that no such substances were permitted to enter from the air. In these solutions the nitro-bacteria oxidized the nitrogen, and grew so markedly that he was able to determine quantitatively and directly their gain in organic substance. Thus he found that in four cultures carried on during about three months the organically combined carbon amounted to (in mg.) :—

No. 11.	No. 12.	No. 26.	No. 30.
19·7	15·2	26·4	22·4

The amount of organic carbon introduced along with the nitro-bacteria was immeasurably small, so that practically all that was obtained must have been produced in the culture.

This very small but absolutely certain increase of organic substance must have taken place at the cost either of the carbon-dioxide of the air or of the carbonate in the solution. As GODLEWSKI showed (1895), this latter source is insufficient, and nitrification does not go on when the carbon-dioxide of the air is shut out. The carbonate further can only be conceived of as a source of carbon-dioxide to the *nitrite*-bacteria, since the nitrous acid which appears must of course decompose the carbonate; in the further oxidation of the nitrite, however, no carbon-dioxide would come off free.

WINOGRADSKY'S quantitative analyses have also proved what had already been suspected by HERAEUS (1886) and HÜPPE (1887), that the *nitro-bacteria are able to form organic materials out of carbon-dioxide*; in other words, they are in this respect, like green plants, *autotrophic*. We have already seen, that the formation of organic material from carbon-dioxide is essentially associated with expenditure of energy, and that the sunlight provides this energy for carbon assimilation in the case of the green plant. The case is different with the nitro-bacteria, for they assimilate the carbon-dioxide in the dark, if only they be provided with ammonia or nitrite, which they oxidize with the aid of oxygen. It would appear that the energy obtained by the oxidation of ammonia takes the place of the energy of sunlight in the case of the green plant, and hence it becomes explicable why WINOGRADSKY found a definite relation subsisting between the amount of organic substances formed and the amount of ammonia oxidized. On an average 35·4 mg. of nitrogen must be oxidized for every mg. of organically combined carbon formed. The individual determinations vary but little from this average :—

Nitrogen oxidized	722·0	506·1	928·3	815·4
Carbon assimilated	19·7	15·2	26·4	22·4
Proportion	33·6	33·3	35·2	36·4

These statements of WINOGRADSKY date from the time when he was unacquainted with the differences between the nitrite- and nitrate-bacteria, and the results he obtained were put down to their collective activity. He would probably have considerably modified his views had he experimented with pure cultures of one or the other, for they exhibit great differences in their capacities for oxidizing nitrogen. In a culture of the nitrite-bacterium the daily amount of nitrogen oxidized gradually rises from 3·0 mg. on the fifth day, to 20 mg. four weeks later, while the most energetic nitrate organism is capable of oxidizing not more than 10 mg. of nitrogen per day. It is only natural that the energy, not only of oxidation but also of assimilation, in the two forms should be quite different.

Further investigations are yet needed to elucidate fully the whole problem. For example, we are as yet quite in the dark how carbon assimilation is effected, and what is the first product of assimilation. It is by no means essential that the process should be carried out in the same way as in the green plant, viz. by the decomposition of carbon-dioxide and the evolution of oxygen. WINOGRADSKY, in fact, has advanced arguments against the possibility of such a method. He observed that if oxygen is given off in this assimilation process, it must be capable of maintaining nitrification just as well as respiration in the green plant may be maintained by the oxygen released in carbon assimilation. WINOGRADSKY, however, did not observe the quantitative relation existing between the nitrogen oxidation and the carbon assimilation in the nitro-bacteria, which differs entirely from that between respiration and assimilation in the green plant; in the case of the nitro-bacteria the

oxygen formed in assimilation is quite insufficient to supply what is wanted for nitrification, while in a green plant assimilation results in the formation of far more oxygen than can be used in respiration. It is *possible* that a splitting off of oxygen from carbon-dioxide and a formation of carbohydrate does occur in the nitro-bacteria, but, according to WINOGRADSKY, urea may also be formed directly by the union of carbon-dioxide and ammonia, from which might arise the other organic compounds found in nitro-bacteria. The behaviour of the nitro-bacteria in presence of urea, however, does not support this hypothesis.

Respiration forms another important problem which still requires elucidation. Do the nitro-bacteria remain content with oxidizing ammonia into nitrous acid, or do they use up organic material they themselves have manufactured? This cannot be easily answered, but it is of interest in relation to our general summary of respiration. PFLÜGER and DETMER's conception of respiration as taking place in the protoplasm, that it is *protoplasm* that *undergoes respiration*, and that the reserves are used to regenerate it has already been discussed (p. 204). This regeneration can be effected by carbohydrate, but not by ammonia. Were it possible to prove that the nitro-bacteria respired no organic material, this hypothesis of PFLÜGER would be definitely established, but it is scarcely possible to bring forward such evidence although it may perhaps be possible to do so in the case of other organisms.

Having now discussed the nitro-bacteria, the relations established for the colourless sulphur-bacteria appear in altogether a new light. Not only are we able to note a perfect analogy between the respiration of ammonia on the one hand and sulphuretted hydrogen on the other, but also the evil effects of supplying organic nutrients to *Beggiatoa*. It is in the highest degree probable (WINOGRADSKY, 1890, p. 275) that both sulphur- and iron-bacteria are autotrophic, and that they grow better when organic materials are completely excluded than when they are present. One wonders why this experiment has not long since been carried out. Then the energy which is obtained by the oxidation of inorganic substances in the case of sulphur- and iron-bacteria is perhaps used up only in the assimilation of carbon, and just as in the case of the nitro-bacteria we have to inquire whether or not a respiration of organic substance takes place here also.

Recently NATHANSOHN (1902) has published some observations on a new group of sulphur-bacteria. Details as to the occurrence of these forms are as yet entirely wanting, and studies on their physiological behaviour require confirmation also. In consequence of their great importance we may add here a few words on the subject of these Bacteria, without waiting for a confirmation of NATHANSOHN's work [OMELIANSKI, 1904]. They do not oxidize sulphuretted hydrogen, but *thiosulphate*, and form therefrom sulphuric acid and tetrathionic acid. Further, they are not sensitive, like nitro-bacteria and *Beggiatoa*, to the addition of organic material, and hence it is possible to prove that they are *entirely unable to oxidize organic substances*, e. g. sugar ; they produce no carbon-dioxide, and *have no normal respiration*. On the contrary, carbon-dioxide is an essential food-stuff, since they form organic substance from it. If NATHANSOHN's experiments are correct, then these forms afford proof of the fact that there are organisms which *respire inorganic material only*.

Let us now return to the nitro-bacteria. The *formation* of organic material out of carbon-dioxide is not the final point of interest in their physiology. It is of importance also to note their behaviour to such organic compounds as are presented to them from without. As we have already remarked, the culture of these microbes was retarded in WINOGRADSKY's experiments by the addition of potassium tartrate and gelatine. A short time previously WINOGRADSKY, in conjunction with OMELIANSKI (1899), investigated the effect of

organic substances in the nitro-bacteria in an exhaustive manner, and their results are summarized (in percentages) in the following table :—

	Nitrite formers.		Nitrate formers.	
Glucose	0·025–0·05	0·2	0·05	0·2–0·3
Peptone	0·025	0·2	0·8	1·25
Asparagin	0·025	0·3	0·005	0·5–1·0
Glycerine	>0·2	—	0·05	>1·0
Urea	>0·2	—	0·5	>1·0
Sodium acetate	0·5	>1·5	1·5	3·0
Sodium butyrate	0·5	>1·5	0·5	1·0
Meat extract	10·0	20–40	10·0	60·0
Ammonia	—	—	0·0005	0·015

In the first column of each series are given the lowest percentages which accelerate development, and in the second the doses which retard it. The symbol > signifies ' more ', but *not much more*, than the dose following.

We may deduce several important conclusions from this table.

1. The different organic substances are by no means *equally valuable* to the nitro-bacteria, but may operate directly as antiseptics. This antiseptic effect is not less than that of carbolic or salicylic acids in the case of ordinary Bacteria. The nitro-bacteria are much more autotrophic than green plants; owing to the fact that the latter are at least facultatively heterotrophic, they *may* get on with organic substances supplied to them from without. We might certainly suppose that the same was true of the nitro-bacteria, if the first assimilation product were known.

2. The very substances which form the best nutrients for ordinary Bacteria and for heterotrophic plants inhibit nitrification most.

3. The nitrite-bacterium is much more sensitive to organic substances than the nitrate-bacterium. On the other hand, the nitrate-bacterium is astonishingly sensitive to ammonia, and that substance is more antiseptic to it than is corrosive sublimate to other organisms.

All these facts are of significance, not only because they disclose to us the remarkable differences and adaptations which occur in the organic world, but also because they explain the rôle of nitrification in nature. The sensitivity of the nitrite-bacterium to organic substances carries with it the conclusion that its development can commence only if all the organic materials which are present in the soil (dead animals and plants, excrement, &c.) have been completely decomposed by ordinary putrefactive organisms, so that the carbon is present as carbon-dioxide, the nitrogen as ammonia, or even as an element. The nitrate microbe is less sensitive to organic compounds, but its development is inhibited by ammonia, and it can develop only after the nitrite organism has operated first. The question now is whether the sharp demarcation of nitrification from fermentation and putrefaction is of significance in organic nature; the answer to this is undoubtedly in the affirmative. In many of the commonest fermentative processes, potassium nitrate is reduced, whereby not only nitrite, but especially free nitrogen is formed in large quantity. If nitrification sets in before the completion of fermentation, the nitrates formed would be denitrified by these ferments instead of being available for the nutrition of the green plant. Such a denitrification does occur under certain conditions, as we shall see in the next lecture. There we shall take the opportunity of studying the *combining* of free nitrogen by organisms in conjunction with its *formation*.

At the present moment we may note that the nitro-bacteria do not confine their activity to arable soil where ammonia is presented to them in the manure, but that they establish themselves on bare rock containing lime, and make use of the traces of ammonia brought down by rain. They then decompose the lime, and thereby render this mineral available for the higher plant as well as by forming nitric acid.

Bibliography to Lecture XVIII.

Baumann. 1888. Landw. Versuchsstationen, 35, 217.
Beijerinck. 1895. Centrbl. Bakt. II, 1, 1.
Beijerinck. 1900. Ibid. II, 6, 193.
Beijerinck. 1901. Ibid. II, 7, 33.
[Delden. 1903. Centrbl. Bakt. II, 9, 81.]
Engelmann, Th. W. 1888. Pflüger's Archiv, 42, 183.
Frankland, G. and P. 1889. Zeit. f. Hygiene, 6, 373.
Frankland, G. and P. 1890. Phil. Trans. Roy. Soc. B, 181, 107.
Godlewski. 1895. Anzeiger d. Akad. Krakau.
Heraeus. 1886. Zeit. f. Hygiene, 1, 193.
Hoppe-seyler. 1886. Zeit. f. phys. Chem. 10, 201.
Hüppe. 1887. Biolog. Centrbl. 7, 701.
[Miquel. 1904. In Lafar's Handb. d. techn. Mykologie, 3, 82.]
Molisch. 1892. Die Pflanze und ihre Beziehungen zum Eisen. Jena.
Nathansohn. 1902. Mitt. d. zoolog. Station Neapel, 15, 655.
Omelianski. 1899. Centrbl. Bakt. II, 5, 473.
Omelianski. 1902. Ibid. II, 9, 63.
[Omelianski. 1904. Lafar's Handb. d. techn. Mykologie, 3, 214 and 234.]
[Rullmann. 1904. In Lafar's Handb. d. techn. Mykologie, 3, 193.]
Schlössing and Muntz. 1877–79. Compt. rend. Paris, 84, 301; 85, 1018; 86, 892; 89, 891, 1074.
Warington. 1888. Centrbl. Bakt. 6, 498.
Winogradsky. 1887. Ueber Schwefelbakterien, Bot. Ztg. 54, 493.
Winogradsky. 1888 a. Bot. Ztg. 46, 261.
Winogradsky. 1888 b. Beitr. z. Morphologie und Physiologie der Bakterien, I; Schwefelbakterien. Leipzig.
Winogradsky. 1890–91. Recherches sur les organismes de la nitrification. Annales Institut Pasteur, 1890: Mitt. I: 4, 213–231; Mitt. II: 4, 257–275; Mitt. III: 4, 760–771; 1891: Mitt. IV: 5, 92–100; Mitt. V: 5, 577–616.
Winogradsky. 1892. Contrib. à la morphologie des organismes de la nitrification (Archives d. Sc. biolog. St. Pétersbourg 1).
[Winogradsky. 1904. Lafar's Handb. d. techn. Mykologie, 3, 132.]
Winogradsky and Omelianski. 1899. Centrbl. Bakt. II, 5, 329.

LECTURE XIX

DENITRIFICATION AND NITROGEN FIXATION. SYMBIOSIS AND METABIOSIS. CIRCULATION OF CARBON AND NITROGEN.

We have already drawn attention on several occasions to the problems connected with the circulation of nitrogen in the organic world. In Lecture XI we established the fact that the green plant supported itself in the first instance on nitric acid, and constructed proteid out of that substance; in Lecture XVII we found that on the death of the green plant the proteid was broken down by micro-organisms in such a way that the nitrogen was finally transformed for the most part into ammonia. When, owing to the combined activity of nitrite- and nitrate-bacteria, the ammonia is changed again into nitric acid, the circle is completed, and the nitrogen once more appears in a form which green organisms can appropriate. The cycle is not, however, quite so simple as this as regards all the nitrogen; a complication appears when in certain processes gaseous nitrogen is *formed*, and when in other cases free nitrogen may be seen to undergo transformation into a combined form. Reference has been already made to these processes, but some of them require further explanation.

We have already seen (p. 219) that free nitrogen as well as ammonia may be produced during the decomposition of proteid. The conditions under which this takes place have not as yet been fully determined, but the process

of denitrification has received considerable attention. In spite of a voluminous literature on the subject (compare LEMMERMANN, 1901, [JENSEN, 1904]) the phenomena of denitrification are in several important aspects still obscure. We speak of denitrification when nitrate is transformed into nitrite, nitrite into ammonia, or finally nitrate or nitrite into free nitrogen. Since we have seen that nitrification, that is, the transformation of ammonia into nitric acid, is a source of energy to the organism, the reverse process can take place only by the *expenditure of energy*, as when free nitrogen is formed from nitrate or nitrite. Therefore denitrification obviously cannot be made to rank alongside of fermentation where gain of energy is the significant point. A certain likeness between fermentation, or, more accurately, *respiration*, and nitrification, is perhaps, however, observable. According to JENSEN (1898, 1899) denitrification occurs only in the absence of oxygen, and the denitrifying Bacteria are anaerobic in the presence of nitrates; when no nitrates, on the other hand, are present they are strongly aerobic. The significance of denitrification rests solely in the fact that there is gain of respiratory oxygen from the nitrates, and this view is supported by an observation of MAASSEN (1901), that substances rich in oxygen, such as chlorates, are able to inhibit the decomposition of nitric acid. Probably these chlorates act in the place of the nitrate, giving up their oxygen, and thus *protecting*, so to speak, the nitrates. Still various investigators have shown that denitrification is also possible in the presence of oxygen, and this fact does not at first sight appear to support the conception of the process just advanced. When we remember, however, that yeast is also incapable of developing alcohol when oxygen is abundantly present, we must admit the possibility of the existence of Bacteria which habitually split off oxygen from nitrogenous compounds, even if it be at their disposal in a free state. It may be concluded from MAASSEN'S researches that certain Bacteria are *always* specifically denitrifiers, while others develop such powers only under definite external conditions. The number of the former type is apparently limited, while the power of occasionally inducing denitrification appears to be widely distributed.

Denitrification is naturally a process of fundamental interest and importance to the agriculturist. In agricultural operations, as we have seen already, large quantities of nitrogen are removed from the land in harvesting, and manuring with nitrogen becomes one of the most necessary conditions of successful agriculture. If this nitrogen be presented in the form of nitrate of potash denitrification must be most carefully guarded against. It is not necessary for us, however, to go into this question more fully; we need only remark that the land would indeed be in a parlous state, were there no process in nature by which denitrification could be compensated for. The reconstruction of nitric acid out of ammonia and nitrites we have already studied in the previous lecture, but the free nitrogen of the air, which, owing to its chemical inertness, is of no service to the organism (compare BUNGE, 1889) is by no means excluded from taking part in the circulation of material in living nature. The combination of free nitrogen has been conclusively proved, and it is both practically and theoretically a phenomenon of the very greatest importance.

Recently J. KÜHN (1901) has provided us with a very interesting proof of nitrogen combination in arable land. He was able to obtain out of a certain field good and even increasing harvests after being sown for twenty successive years with winter rye, *without any nitrogenous manuring whatever*. This showed that more nitrogen was annually combined in the soil than was removed in the process of harvesting; and since the rye is itself incapable of bringing about such a combination, and since further the amount of combined nitrogen precipitated on the soil nothing like makes up for the loss by harvesting (compare p. 136), obviously atmospheric nitrogen must have been combined to a very large extent in the *soil*. We have again to thank WINOGRADSKY for a

very thorough elucidation of the details of the part played by certain Bacteria in this process, BERTHELOT (1892) having previously shown that nitrogen combination must be due to the activity of Bacteria.

WINOGRADSKY (1895) made use of the experience he had gained in cultivating sulphur- and nitro-bacteria, and began his studies on the organism which combined nitrogen by preparing a nutritive solution, which, in addition to dextrose, contained the usual salts, save that no nitrogen compounds were present. He hoped by this 'selective' mode of culture to provide the organism he was in search of with all the conditions it required, and so isolate it, by shutting out ordinary soil-bacteria which were unable to grow save in the presence of combined nitrogen. His expectations were not disappointed. After the culture fluid had been placed at the bottom of a glass jar and inoculated with a small quantity of arable soil, a vigorous formation of butyric acid soon began to take place, and irregularly spherical masses of zoogloeae made their appearance. When the acid was neutralized, the fermentation continued without intermission until the whole of the sugar was used up. Apart from the products of fermentation, the fluid had undergone essential alterations, as was shown by the fact that when fermentation had finished, Fungi appeared on the zoogloeae, and that after these had destroyed the butyric acid, Algae introduced themselves. None of these organisms could exist in the original fluid, seeing that it was destitute of combined nitrogen; their appearance after fermentation demonstrated the fact that combined nitrogen was present afterwards, and chemical analysis confirmed this.

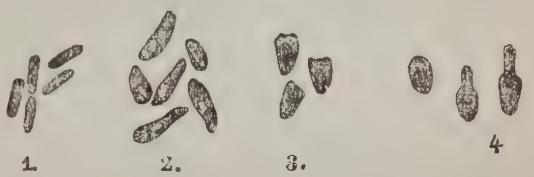


Fig. 41. *Clostridium pasteurianum.* *1*, Vegetative rods. *2*, Sporogenous spindle-shaped rods. *3*, Burst spindles with spores. *4*, Germinating spores. (After WINOGRADSKY.) From FISCHER (Vorles. ü. Bakt. 2nd ed.).

Microscopical investigation of the zoogloeae disclosed the presence of two filamentous Bacteria, and a species of *Clostridium* (i. e. a bacterium which swells into a spindle form when spore formation takes place). Isolation and cultivation of the filamentous Bacteria was easily effected, and it was found that they were ordinary saprophytic forms which required extremely little nitrogen, but were quite unable to cause it to enter into combination; moreover, they were found not to be the cause of butyric acid fermentation. Interest thus became concentrated on the third form, *Clostridium pasteurianum*, which, morphologically, must be ranked along with the butyric acid Bacteria previously referred to, capable like them of inducing butyric acid fermentation, but which differs greatly from them in its behaviour to nitrogen. The isolation of *Clostridium* presents many and great difficulties, and is only successfully accomplished if it be sown on carrots in vacuo. If a pure culture on this medium be once more placed in the original non-nitrogenous nutritive solution, fermentation and nitrogen combination do not take place. Both processes commenced at once when WINOGRADSKY added the two bacterial forms present with *Clostridium* in the zoogloeae, or when he excluded oxygen entirely. The significance of the three Bacteria was thus explained. When alone, *Clostridium pasteurianum* is able to combine nitrogen; it is strongly anaerobic and thus, in a pure culture, is capable of growth only when oxygen is quite excluded. In nature, however, it is able to live in the aerated upper regions of arable soil if the two other Bacteria associated with it protect it from the action of oxygen. These two Bacteria fulfil no specific function in themselves; they may be replaced by other appropriate organisms which consume oxygen, e. g. Hyphomycetes. Not every organism, however, is

capable of fulfilling this protective rôle in the same way. The organism must *first* operate on the culture medium by absorbing the oxygen before *Clostridium* can begin to combine the nitrogen ; the protecting organism must also obtain combined nitrogen in the first instance from the nutritive solution ; later on *Clostridium* provides for it in this respect. It would thus appear that organisms which make very small demands on nitrogenous compounds will be the most suitable forms to accompany *Clostridium*. This condition is fulfilled by the two Bacteria present in the zoogloeae because such traces of nitrogen as are unavoidably present as impurities in the reagents employed are sufficient for the purpose, although WINOGRADSKY showed that a minute addition at first of ammonia or nitric acid induced much more rapidly both fermentation and nitrogen combination.

Working on his previous experiences, WINOGRADSKY has since then been able to isolate *Clostridium pasteurianum* by a second method much more rapidly and effectively. He added a trace of garden soil to a non-nitrogenous nutritive solution, and allowed a stream of nitrogen gas to pass through the fluid. A drop of this solution was after a certain time transferred to a fresh nutritive solution, identical in character, and this process was repeated several times. The final culture was heated to 80° C. after *Clostridium* had formed its spores, so that all admixtures of foreign organisms were killed. The result was a pure culture of the spores of *Clostridium*.

How nitrogen assimilation is carried out in this case is, however, quite unknown. We know neither the primary nor the final products of assimilation ; we do not know whether ammonia is formed and made further use of or whether a complicated nitrogenous substance, e. g. some kind of proteid, arises at once. One of WINOGRADSKY'S researches on this subject shows that the nitrogen occurs chiefly in an insoluble organic condition, and only in small quantities as soluble compounds in the nutritive solutions. The latter, which possibly becomes free only when the *Clostridium* cells die, serves as a nutrient for other organisms, especially for the two concomitant Bacteria.

Clostridium pasteurianum is an anaerobe. It acts fermentatively on cane sugar, dextrose, laevulose, and certain other carbohydrates ; but it is *unable* to make use of starch, cellulose, lactose, and higher alcohols. According to WINOGRADSKY (1902), the products of fermentation are, on the one hand, butyric and acetic acids (about 45 per cent. of the sugar), and carbon-dioxide and water on the other (about 55 per cent. of the sugar). The fermentation acts as a source of energy, especially for the purpose of combining atmospheric nitrogen, and so we cannot wonder that WINOGRADSKY succeeded in establishing quite definite numerical relations between the amount of sugar used up, and the gain in nitrogen (viz. 1 g. of sugar fermented for every 2·5–3 mg. of nitrogen combined), but he was unable to say whether the sugar was employed only as *material for fermentation*, or whether it was also *nutritive*. There is no evidence at present available, however, to show whether *Clostridium* withdraws not merely nitrogen but also carbon from the air, or whether it assimilates carbon-dioxide like nitro- and sulphur-bacteria.

BERTHELOT had imagined that the capacity for fixing free nitrogen was possessed by *many* micro-organisms, WINOGRADSKY finds it limited to *Clostridium* and forms related to it ; at least these forms are the only ones which are able to commence and carry on vegetative growth without combined nitrogen. Following on WINOGRADSKY'S researches, it has often been stated that other organisms also are able to make use of atmospheric nitrogen. Thus BEIJERINCK (1901) observed a bacterium (*Azotobacter*) of unusually large size, which forms no spores, and which on that account could not be isolated by WINOGRADSKY'S method ; it develops on organic nutrients in presence of air, especially on mannite, propionic acid, &c., without any combined

nitrogen, since it at first makes use of the traces of combined nitrogen, occurring as impurities in the nutritive solution, and afterwards assimilates the nitrogen of the air. According to the same authority, there are certain Cyanophyceae (*Nostoc*, *Anabaena*) which behave in a precisely similar way to *Azotobacter*, so that BEIJERINCK has been led to establish a special physiological class of organism, to which he applies the term 'oligonitrophilous', among which he reckons *Clostridium*. We must note in this relation that there is one great difference between BEIJERINCK'S and WINOGRADSKY'S researches, for the latter has proved the occurrence of a nitrogenous gain by quantitative chemical analyses, whilst the former omits all such proof. More recently WINOGRADSKY (1902, Centrbl. Bakt. II, 9, 43) has taken the same view of BEIJERINCK'S work, and BEIJERINCK himself (1902, Centrbl. Bakt. II, 9, 3) admits that *Azotobacter* does not assimilate free nitrogen. His more recent statements as to the real nitrogen-combining Bacteria must therefore be received with a certain amount of scepticism. [It has been shown more recently still that *Azotobacter* does assimilate free nitrogen; the essential difference between *Azotobacter* and *Clostridium* lies in the fact that the former is aerobic (compare the comprehensive exposition of the subject by KOCH, 1904). Both forms occur in the sea as nitrogen combiners (KEUTNER, 1904).]

The combination of atmospheric nitrogen has been maintained for other forms, e.g. for *Aspergillus* and *Penicillium* by PURIEWITSCH (1895), and for other Mould-fungi by SAIDA (1901). The increase in combined nitrogen in the cultures is, however, very minute, and we must await confirmatory experiments before we can pronounce definitely on the subject. CZAPEK (1902, Beitr. z. chem. Phys. u. Path. 2, 559) was unable to observe any combination of free nitrogen in any of his experiments with *Aspergillus*. The power of combining the free nitrogen of the air is even more doubtful in the case of the endosporal bacillus (*Bacillus ellenbachensis*) than in the case of the Fungi referred to; accurate investigations have indeed shown that such a capacity does not exist. In spite of this, pure cultures of this bacillus are sold under the name of 'alinite' for impoverished soils; they are said to aid in the combination of nitrogen in the soil when spread over the ground.

If as vigorous a combining of nitrogen took place in all soils as in KÜHN'S experiments mentioned at the beginning of this lecture, neither alinite nor any artificial manuring with nitrogen would be necessary. Experience teaches us the contrary, however; generally speaking, nitrogenous manuring is indispensable, and the Leguminosae only form an exception, to be considered afterwards. The combining of nitrogen in KÜHN'S fields must have been unusually vigorous, and for this there must have been some special reason. If we assume that *Clostridium pasteurianum* was the active micro-organism concerned, the carbohydrates necessary for its support must have been present in special abundance, so that we are met by a problem which has not as yet been considered, viz. as to how *Clostridium* is able generally to procure sugar in nature. Two sources seem possible, one from the deciduous parts and remains of cultivated plants, the other from the lower Algae, which always occur in the soil. It has been clearly established that combination of nitrogen takes place remarkably well in soils which are rich in Algae, from which one may conclude that the Algae and *Clostridium* stand in intimate relations to each other, the Algae obtaining combined nitrogen from the *Clostridium*, the *Clostridium* receiving soluble carbohydrates from the Algae (KOSSOWITSCH, 1894).

As already remarked, the Leguminosae play a special part in agricultural processes, not only because they grow in sterile sandy soils without any addition of nitrogenous manure, but because they actually improve such soils and make them suitable for the growth of plants which do not belong to that family. These peculiarities of Leguminosae were known, in part at least, to the ancients

(PLINY, Historia Naturalis, vol. 8; cited by JACOBITZ, 1901), but were for the first time clearly comprehended by SCHULTZ-LUPITZ (1881), who obtained fifteen crops of lupins one after the other from sandy soils on his estate merely by mineral manuring without any nitrogen, and without noticing any diminution in their yield. He also noticed that, after a crop of lupins, the cereal harvest was doubled or tripled in amount. SCHULTZ did not remain content with these results; he made an analytical estimate of the nitrogen in the soil, with the following result :—

1. Land which had neither been manured or cultivated for fifteen years and which had been used as sheep pasture contained 0·027 per cent. of nitrogen in the arable layers to 6″ deep, and 0·21 per cent. in the subsoil from 6″ to 24″ deep.

2. The same soil after being cultivated for fifteen years with lupins and manured with minerals only, gave to 8″ deep 0·087 per cent., and from 8″ to 24″ deep, 0·025 per cent.

It will be noticed that a very apparent gain in nitrogen had been effected in the upper soil layers, and this was confirmed later by FRANK (1888) when he examined the same fields after twenty years of lupin culture.

Both husbandman and agricultural chemist may therefore conclude from the statements of SCHULTZ that the Leguminosae, and especially lupins, must have the power of combining atmospheric nitrogen. Botanists, on the other hand, may refer to an experiment of BOUSSINGAULT'S, of the accuracy of which there can be no doubt, which shows that peas and lupins, which germinate in soils containing no nitrogen, and which have access to no other source of nitrogen than that present in the air, exhibit neither gain or loss in their nitrogenous contents after a long continued experiment. Exact though this experiment may be, it in no way demonstrates the inability of Leguminosae to combine nitrogen *in general*, but only under the conditions of this experiment. BOUSSINGAULT'S experiments are in reality not antagonistic to the classical researches of HELLRIEGEL and WILFARTH, although these latter authors have advanced *definite proof of the power of the Leguminosae to combine free nitrogen.*

HELLRIEGEL and WILFARTH (1888) used as a culture medium very pure quartz sand, which was completely sterile, save for the addition of minerals. The Leguminosae experimented with were compared most carefully with cereals (oats and barley), which are known to be incapable of growth in the absence of nitrates and other sources of nitrogen present in soils, and which also have been specially shown to be unable to make any use of the nitrogen of the air.

Certain Leguminosae (*Serradella*, peas and lupins) were planted in this sand, after it had been freed from all micro-organisms by heat, i.e. sterilized, and kept free from them, and an experiment was carried out in precisely the same way as with cereals, when growth took place only on the addition of nitrates. This agrees with the behaviour of the Leguminosae in BOUSSINGAULT'S experiment referred to above. An important difference was noticed, however, as soon as a small quantity of an extract of arable soil had been added to the sterile soil free from nitrogen; then the crop showed a remarkable gain in nitrogen, which could only have arisen from the employment of atmospheric air. A numerical illustration will make this clearer (HELLRIEGEL, 1888, p. 145):—

	Without addition of soil extract.		With addition of soil extract.	
	Dry weight.	Gain in N.	Dry weight.	Gain in N.
	g	g	g	g
Serradella	0·092	−0·022	16·864	+0·326
Lupins	0·919	−0·049	44·718	+1·077
Peas	0·779	−0·025	17·616	+0·449

If the experiments are carried out with oats, instead of with Leguminosae, the addition of the soil extract produces no result.

The effect of the soil extract cannot be due to the amount of nutrient

which it contains, but to the activity of the micro-organisms present, since on heating it to a temperature of 70° C., its effect is at once neutralized. That it is not due, however, to the presence of some kind of nitrogen-combining micro-organism in the soil is shown by the absence of any result in the case of cereals. The organisms must be such as have special relationships with the Leguminosae. Further, *different* Leguminosae require *different* micro-organisms, since the extract of a soil in which beetroot has been grown in which peas and various species of clover had been cultivated for a long time up to the fruiting stage, but in which no *Serradella* or lupins had ever been grown, furthered the growth of peas only, but not of *Serradella* and lupins. Hand in hand with this stimulating influence of the soil extract goes the formation of special nodules on the roots of Leguminosae, whose existence had been long known, but as to whose origin and nature no explanation was forthcoming.

HELLRIEGEL and WILFARTH showed in the clearest way that the micro-organisms present in the soil extract were also the cause of the formation of the nodules on the roots of Leguminosae, and that these plants could assimilate atmospheric nitrogen only when the micro-organisms were present in the root nodules. 'To show that the Leguminosae make use of free nitrogen for nutritive purposes', write HELLRIEGEL and WILFARTH, 'it is not sufficient merely that any kind of lower organism should be present in the soil, but it is essential that *certain species* of the latter should first of all enter into *symbiotic relation* with the former.'

Following DE BARY (1879), we may define symbiosis as a partnership of two organisms of such a nature that both receive benefit by living together, or where at least the benefits are not all on one side. In the latter case, one would have to speak of it as parasitism. HELLRIEGEL and WILFARTH have not, however, explained in individual cases wherein lies the reciprocal benefit to the leguminous plant and to the Bacteria which live with it, and this has not even yet been made clear, in spite of the series of important memoirs by BEIJERINCK (1888), PRAZMOWSKI (1890–91), FRANK (1890) and others on the formation and significance of the nodules.

Without going into details, we may present here the essential results of recent investigations on the leguminous nodules by a reference to Figs. 42 and 43. When certain motile rodlike Bacteria, known collectively as *Bacterium radicicola*, have entered the roots of Leguminosae, they increase there to an almost astonishing degree. Just as we have seen in the case of galls, the Bacteria stimulate the cells of the root and produce local hypertrophy, so that we may term these nodules 'bacterium galls'. In the majority of the cells of the nodule one finds masses of *Bacterium radicicola*, which later on degenerate in peculiar ways, and exhibit large spherical or branched 'involution forms', rich in proteid. These involution forms ('bacteroids') are then used by the leguminous plant as proteid reserves, that is to say, they are broken down and employed in the formation of fruit. Only part of the Bacteria become altered into bacteroids, and are sacrificed for the good of the leguminous plant, the rest persist, and, after the destruction of the nodule, remain in the soil, and serve to infect the new Leguminosae of the next year.

Bacterium radicicola has been cultivated apart from the plant in suitable nutritive media by several investigators, and the strongest possible proof is forthcoming that the bacterium is the cause of the nodule formation. The conditions of life of this organism have been established in the clearest possible way, and we have to thank MAZÉ (1897) especially for proof that nitrogen is combined by its means. Similar evidence was also furnished by other authorities, but they provided *Bacterium radicicola* with ammonia or with no *combined* nitrogen at all, in the expectation of proving even more clearly that a combination of nitrogen took place. BEIJERINCK employed asparagin, and thought

that he noticed a gain in nitrogen, MAZÉ, finally, used proteid, believing that the Bacteria in the first instance, doubtless, obtained it from the plant. His results were striking :—

	I.	II.	III.
Nitrogen in the culture solution	62·1 mg.	70·7 mg.	22·4 mg.
Nitrogen at the end of the experiment	102·9 ,,	118·2 ,,	45·8 ,,
Gain in nitrogen	40·8 mg.	47·5 mg.	23·4 mg.

[MAZÉ's results have been questioned by HILTNER (1904).]

In addition to nitrogen in the form of proteid, *Bacterium radicicola* uses up very considerable quantities of sugar, and we must conclude that both

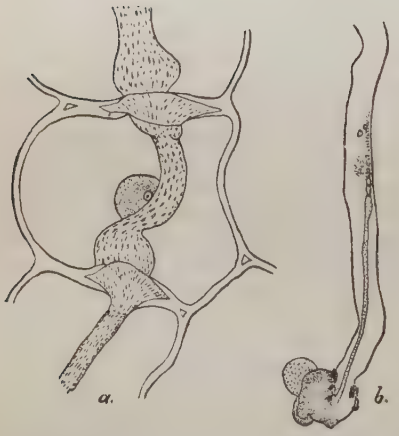


Fig. 42. Entry of Bacteria into the roots of Leguminosae. *a*, cortical cells of the root of the pea, containing tubular masses of Bacteria, × 650 ; *b*, roothair of the pea, on the left of which a number of Bacteria have accumulated outside. Apically the Bacteria are seen mixed with the protoplasm of the hair, whence the infecting threads are proceeding inwards to form the tubular bacterial aggregates, × 175. From FISCHER (Vorles. ü. Bakt. 2nd ed.).

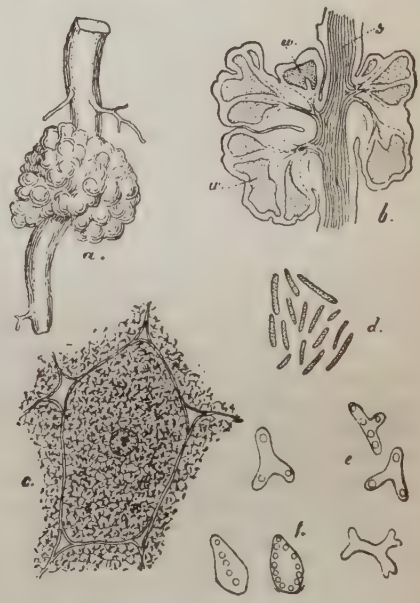


Fig. 43. Root nodules of Leguminosae. *a*, root nodule of lupin, nat. size ; *b*, longitudinal section of the same, several times magnified (*g*, vascular bundle ; *w*, bacteroidal tissue); *c*, bacterial cells of lupin (the Bacteria are indicated as black dots, × 600) ; *d*, Bacteria of lupin ; *e*, bacteroids of *Vicia villosa* ; *f*, bacteroids of *Lupinus albus* ; *d-f* × 2500. From FISCHER (Vorles. ü. Bakt. 2nd ed.).

substances are provided by the leguminous plant, sugar continuously and proteid only at the beginning, since obviously the fixing of atmospheric nitrogen commences after a certain time.

Since, in the end, the bacterium yields nitrogen to the leguminous plant, and the latter yields carbon in an appropriate form to the bacterium, we may speak of the union of the two organisms as a case of symbiosis. It is true the association has been otherwise interpreted ; FISCHER (1903), for example, considers the leguminous plant as a parasite on the bacterium, but this does not appear to us to be a correct view to take.

It is quite probable that in the foregoing exposition of the phenomenon of nitrogen assimilation in Leguminosae there will be found many omissions. Indeed the researches of HELLRIEGEL and WILFARTH prove only *indirectly* that the nitrogen of the air is combined ; they show a gain in nitrogen, and demonstrate that the air is the only source whence that element could have

been obtained. Such being the state of the case, it is of interest to note that *direct* evidence of the fact has been advanced by SCHLÖSSING and LAURENT (1890) who have calculated how many milligrams of nitrogen a pea takes from the air during the several months of its vegetative growth, checking this result by estimating the increase in nitrogen in the soil and in the crop. The following summary shows that the agreement between these calculations is almost perfect :—

Atmospheric nitrogen introduced into the culture vessel	2681·2 ccm.
" " withdrawn from " "	2653·1 "
Amount of nitrogen assimilated	29·1 ccm.
	= 36·5 mg.

Nitrogen in soil and seed	32·6 mg.
" " " crop	73·2 "
Nitrogen assimilated	40·6 mg.

Another point of interest is that demonstrated by NOBBE and HILTNER (1899 b), that the atmospheric nitrogen is combined in the *nodules* and not at all in the *leaves* of the nodule-bearing plant. It was previously thought that the leguminous plant in presence of *Bacterium radicicola* became altered in some way so that it acquired the power of fixing free nitrogen. NOBBE and HILTNER infected plants of *Robinia* with *Bacterium radicicola*, growing them in a culture fluid from which nitrogen had been excluded, and observed the formation of nodules under water ; but so long as these remained immersed they were found to be useless to the plant, and it was only when they were brought into the air that they commenced to assimilate the nitrogen. These facts prove that the nitrogen must enter the *nodules themselves* if it is to undergo fixation.

Finally, we may note that many authors have been successful in seeing the evolution of the Bacteria into bacteroids in artificial nutritive solutions (BEIJERINCK, 1888; HILTNER, 1900; STUTZER, 1901). Nevertheless it has not as yet been explained why only *certain* and not *all* Bacteria are transformed into bacteroids in the plant, a phenomenon which is of the utmost importance in relation to the conservation of *Bacterium radicicola* in nature (p. 240).

If we describe the association of Leguminosae with nodule bacteria as a case of symbiosis, then, looking backwards, we may also term the relationship of *Clostridium pasteurianum* to the two associated bacteria as symbiosis also, and we may take this opportunity of drawing attention to some other cases of the same kind. Beginning with *Elaeagnus* and the alder, we find that both these trees produce on their roots nodules which remind us of those of the Leguminosae, and which seem to carry out similar functions. HILTNER (1896) has shown in the case of the alder that when the nodules are absent it can develop only if nitrogenous compounds are provided, but that after the nodules have been formed the nitrogen of the air is sufficient. [HILTNER gives a very instructive illustration of alders, which have been grown in sand destitute of nitrogen, one with and one without nodules (HILTNER, 1904, p. 63).] Similar conditions obtain in *Elaeagnus*, but research is required as to the mode of development of the nodule-forming organisms in both cases ; recently SHIBATA (1902) has supplied us with an interesting account of their structure.

Seeing that the use of free nitrogen by higher plants is not confined to the Leguminosae, we are lead to believe that FRANK'S (1890) view is the correct one, and that this power may be possessed by *all* plants to a greater or lesser degree. It will be sufficient to say on the other hand that careful experiments made on the majority of Phanerogams, e. g. on Gramineae and Cruciferae (AEBY, 1896, PFEIFFER and FRANKE, 1897) have given only negative results. In other cases, however, and especially in such as exhibit a symbiotic union between

Fungi and Phanerogams, nitrogen fixation is a proved fact, or, at least, very probable. One of the Coniferae especially, *Podocarpus*, is known to possess nodules on its roots. These nodules are modified lateral roots, whose tissues have become filled with the hyphae of a fungus. *Podocarpus* cannot be cultivated in the absence of this fungus, but NOBBE and HILTNER remark (1899 a) that they were able to grow *Podocarpus* with perfect success for five years in quartz sand, from which nitrogen was entirely absent. There can be no doubt that this plant is able to fix atmospheric nitrogen, and it is extremely probable that the fungus plays an important part in the process. More recently, HILTNER (1899) has studied *Lolium temulentum*, with which, according to VOGL and NESTLER, there is constantly to be observed associated a fungus growth. HILTNER's observations render it probable that here also fixation of nitrogen takes place, and it may be concluded that many parasitic Fungi act in the same way. HILTNER thought that the *luxuriant growth* of many plants attacked by Fungi was evidence in favour of his view, but BREFELD (1902) has shown that this does not apply to Ustilagineae, although it cannot be said that that is the case with other Fungi.

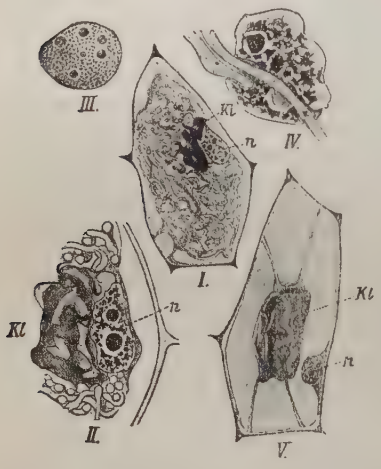


Fig. 44. *Psilotum triquetrum*. *I*, Cell of the root filled with fungal hyphae; *n*, nucleus; digestion of the fungus beginning at *Kl*; × 210. *II*, nucleus, *n*, and digested fungus, *Kl*, in a similar cell, × 400. *III*, Normal resting nucleus from the rhizome of *Psilotum*, × 625. *IV*, nucleus of an infected cell, × 625. *V*, Cell completely digested by the fungus, *Kl*, the digested fungal mass enclosed in a cellulose membrane. × 240. After SHIBATA (1902).

The so-called 'mycorhiza', a symbiotic union of a fungus with phanerogamic roots, is of wide occurrence, and first suggests itself to us in this relation. There are two forms of this union, endotrophic and ectotrophic. The former has been long known, in fact since the time when SCHLEIDEN pointed out its occurrence in *Neottia nidus avis*, and its widespread distribution has been demonstrated by FRANK (1887) and SCHLICHT (1889), especially in Orchidaceae, Ericaceae and Epacridaceae. These Fungi, which have as yet been very little investigated from the systematic point of view, enter the cells of the root, and increase there without killing the cells of the host. *Neottia* has been carefully investigated by W. MAGNUS (1900). In this case the fungus enters from without, and branches at some distance from the epidermis, so as to completely fill up a series of concentric layers of cells in the root and rhizome. The cells in which the fungus lives do not all behave in the same way. In certain cells the fungus grows vigorously, and on the death of the protoplasm of the host, forms organs for the purpose of maintaining its existence over winter, and for the infection of newplants the following year. The fungus is partly digested in other cells, on the other hand, and its abundant proteid constituents go to the nourishment of the host, while the indigestible portions are collected into a ball in the centre of the cell, and are there enclosed by layers of cellulose. The same thing is seen in *Psilotum triquetrum* (SHIBATA, 1902), only in this case the fungal host-cells and the digestive-cells are arranged in no definite order. In the host-cells the fungus hyphae are confined to the periphery of the cell, and the nucleus undergoes no alteration; in the digested cells, on the other hand, one finds (Fig. 44) a dense ball of hyphae, which, beginning at one point, is gradually disorganized (*I* and *II Kl.*), while at the same time the nucleus increases greatly in size, and undergoes special internal altera-

tions as well (compare Fig. 44, *III* and *IV*). Finally, the indigestible portion of the fungal mass aggregates into the centre of the cell (*V*), where it becomes surrounded by a membrane. According to SHIBATA, there is only one slight difference between *Psilotum* and *Podocarpus*; the cells of the *Podocarpus* nodules are densely filled with hyphae, and these are digested. Since the assimilation of free nitrogen very probably takes place in *Podocarpus*, we shall not be far wrong in interpreting the mycorhizal condition in other plants in the same way. [TERNETZ (1904) has managed to isolate a fungus from the roots of Ericaceae, which is in all probability the one concerned in the formation of the mycorhiza, and which is the active agent in assimilating the atmospheric nitrogen.] The knowledge we have gained as to the phenomenon in *Neottia* and *Psilotum* renders the various relations of Bacteria to Leguminosae all the more intelligible (compare p. 239).

Accepting this interpretation of endotrophic mycorhiza, the question comes to be whether a combination of two *non-chlorophylliferous* organisms, e. g. *Neottia* and a fungus, can be termed a case of *symbiosis*, since it is not readily comprehensible wherein the reciprocity can lie; our knowledge of the nutritive relations is still too inadequate, and hence we may postpone further discussion of the question. If the Phanerogams possessing mycorhiza be green and can therefore assimilate carbon, it may be assumed that the activities of the two symbionts are so regulated that the fungus collects the nitrogen and the higher plant the carbon.

At the same time there may be another explanation of this association, other than the fixation of atmospheric nitrogen, viz. that the higher plants are *peptone* or *asparagin* organisms, and that the duty of the fungus is to *manufacture* these nitrogenous compounds out of *humus*. Possibly, however, the fungus aids in the absorption of *materials of the ash*, and does not supply the needs of the higher plant for *nitrogen* at all (STAHL, 1900). Fungi make very heavy demands on such materials, and since they collect these very rapidly, they are vigorous competitors with Phanerogams which work more slowly on soils poor in nutritive salts. Higher plants are able to grow far better in humus which has been deprived of the Fungi naturally present. As long as these Fungi are present the Phanerogams exhibit all the evidences of 'mineral starvation'. A mycorhizal union occurs especially in such plants as live in humus, or for other reasons exhibit feeble inflow of minerals (e. g. weak transpiration). Hence STAHL assumes that these plants make the Fungi contribute to their wants in that respect, turning antagonistic neighbours into efficient assistants. The part played by the higher plant so far as carbohydrate is concerned, becomes intelligible on this view. On the other hand, STAHL's hypothesis appears to us to be subject to criticism, in that the fungus lives in most cases quite in the interior of the root, and hence cannot be in a very suitable position to aid in *absorbing* nutritive salts from the soil. The function of the fungus, however, according to STAHL, consists not merely in the *absorption* of the nutritive salts from the soil, but also in their *transformation*, so that the other member of the symbiotic union may receive the products of assimilation ready made. STAHL comes to this conclusion from noting that the majority of 'mycotrophic' plants do not contain in their tissues certain waste bodies, such as calcium oxalate, which are associated with the assimilation of nutritive salts (comp. pp. 141 and 197).

We must now glance at *ectotrophic* mycorhiza. This form of mycorhiza was first drawn attention to by KAMIENSKI (1881) in *Monotropa*, and soon afterwards FRANK demonstrated its occurrence in a large number of our forest trees (Cupuliferae, Betulaceae, Coniferae). The Fungi—apparently members of the Agaricinae and Tuberaceae—generally do not enter in this case into the cells of the roots, but form a densely-woven layer covering the root, not even leaving the growing points free. Here and there single fungus cells enter in between the super-

ficial cells of the root, but these are always confined to the intercellular spaces. This association is accompanied by certain differences in form and anatomical structure in the root, which render the presence of the mycorhiza easily recognizable, and which must have a physiological significance. One of these differences is the non-formation of roothairs on roots provided with fungus mycelium (Fig. 45), so that the absorption of all nutrients and water is possible only through the medium of the fungus. From what has been already said as to the small amount of nitrogen in woodland soils (p. 133), one can only conclude that the Fungi but not the trees are restricted to ammonia; but we are ignorant what the nitrogen compounds are which the root receives from the fungus covering. STAHL's hypothesis would seem at first sight to be much more applicable to this type of mycorhiza than to the endotrophic form. On further reflection, however, difficulties appear here also. If the fungus actually anticipates the tree in acquiring mineral materials from the soil, why does it not retain them? Why does it, after assimilating them, give them up again? In the endotrophic mycorhiza this is explained by the fungus being digested, but there is no reason to believe that a similar process takes place in the ectotrophic type. Several problems await solution here, and it is astonishing how little experiment teaches us in this relation. And yet systematic experiments are not to be considered as hopeless; we know how NOBBE (1899) managed to develop pines, firs, larches and beeches with perfect success for a period of twenty-five years in pure quartz sand, free from humus. At all events, there appeared to be no hereditary fixing of the adaptation, and hence experiments are not hopeless. According to MÖLLER's (1902) researches, the significance of ectotrophic mycorhiza has again been called in question [since the Brandenburg pine has all the less mycorhiza the more humus the soil possesses]. It is to be expected, however, that these experiments will show that among mycotrophic plants, in addition to real cases of symbiosis, there are associations which are harmless and perhaps of no importance at all, and others which are really cases of parasitism; it may be that the *fungus* is the parasite, and again it may be that the *seed-plant* is the parasite. In many endotrophic mycorhiza on green plants the *fungus* must be the parasite, among the ectotrophic types on colourless plants (e. g. *Monotropa*) it must be the *seed-plant* that is parasitic.

Fig. 45. *I*, Beech root from woodland humus (magnified); *p*, fungal cords, united with soil particles at *a*. *II*, Beech root from sterile humus (magnified); *c*, rootcap; *h*, roothairs. (From DETMER'S Smaller Practical Botany.)

In speaking of symbiosis, it is impossible to ignore the case of lichens, to whose very remarkable association of fungus and alga DE BARY (1879) first applied the term symbiosis. The conception of a lichen as a combination of alga and fungus types was first enunciated by SCHWENDENER (1869, Algentypen der Flechtengonidien. Basel). On this question DE BARY (1865, Morph. u. Phys. d. Pilze, Flechten, &c.), and also REINKE (1894, Jahr. f. wiss. Bot. 26, 524) should be consulted. Notwithstanding the work which has been accomplished, we do not even yet completely understand the *modus vivendi* of this symbiosis. BEIJERINCK (1890) and ARTARI (1899) have shown that certain lichen-Algae are 'peptone plants', and we may suppose that the fungus contributes peptone to the combination, while it is naturally the business of the alga to assimilate

carbon-dioxide, supplying carbohydrate to the fungus. It is possible that we have here a case of true parasitism. Not every parasite treats its host plant so harshly as to kill it either wholly or partially; the craftiness of the parasite lies in this, that it keeps its attack within bounds such that the life of the host is not imperilled, permitting in this way a longer period of usefulness and advantage on the part of the host. Reference should be made here to the Uredineae and the Peronosporeae. In the case of lichens, however, one sees not only that no damage is done to the algal cells, but that on the contrary the fungus seems to stimulate them to further development; the algal cells are larger in the lichen than they are in the free condition. Still, that fact does not negative the idea of parasitism, for in other cases we have seen that an increase in size of the host-cells takes place owing to the stimulating effect of parasitic Fungi; and we are able to explain the effect in some measure by remembering that well-known poisons administered in small doses are capable of acting as developmental stimuli.

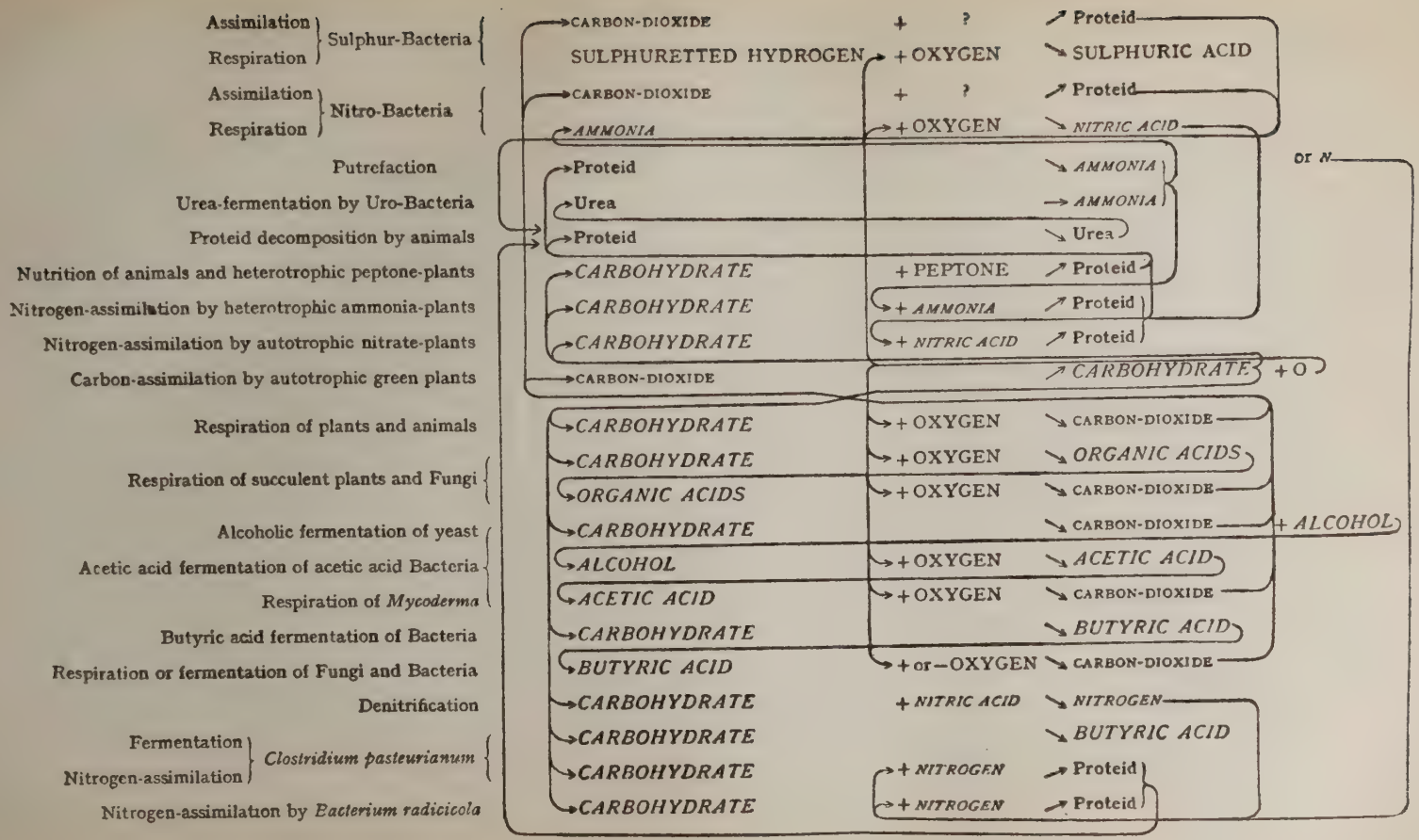
It is not within our power to enter into the numerous cases which have with greater or less justice received the name of symbiosis. We may, however, conclude that the term symbiosis may be applied in a more extended sense. Symbiosis in the most restricted sense may be applied to a single organism formed by the coming together of two symbionts, the combination possessing certain constant functional peculiarities, which the constituents do not exhibit or exhibit in a less degree (lichens, Leguminosae); on the other hand, to a certain extent, we may speak of symbiosis when both symbionts are united only in so far as, e. g. *Clostridium pasteurianum* is with the two aerobic Bacteria, in which case they form an amorphous zoogloea; and we may still speak of symbiosis when green Algae and *Clostridium* act in *conjunction* with each other in arable soil, and *assist* each other with their metabolic products. If we go a step further, we meet with organisms which appear in the same situation, but *following* each other, one preparing the soil for the other; this phenomenon we may term *metabiosis* (WARD, 1899). The close relationship between symbiosis and metabiosis is obvious. We have already drawn attention to the wide distribution of metabiosis, and we may best express our views as to plant metabolism by arranging in tabular form a review of the metabiosis of the various organisms of which we have spoken. We shall limit ourselves in general to the two elements which have played the chief part in our discussion, to the study, that is to say, of the circulation of carbon and nitrogen in the organic world. Chemistry tells us that no substance on our planet is ever *lost*; but that an active *circulation of matter* takes place on the earth, a circulation which is closely connected with metabiosis. Every organism standing by itself would soon have caused alterations in the outer world, which would have rendered continued existence impossible. It is only by the existence of numerous organisms with diversified functions that the perpetual renewal of life on the globe is possible.

The following table makes no claim to completeness; even what it does show is not perfect, since there are many metabolic products, which, if indicated by arrows, would detract from the clearness of the diagram. The carbon-assimilation of green plants is indicated in the middle of the table, and for this the sunlight provides the requisite energy. On the storage of solar energy in the carbohydrates are indirectly based all the processes which are referred to in the summary. In the separate metabolic processes only those products of metabolism which are of especial interest at the moment are indicated. Chemical equations are omitted; the outgoing and the final products of a process are indicated by arrows which give also the direction of the course of the reaction. If an expenditure of energy is necessary for the reaction, the arrow is directed upwards; a *gain* in energy is shown by a downwardly directed arrow; when the arrow is horizontally placed, it indi-

cates a splitting without any actual change in energy. Oxygen is printed in large Roman capitals, carbon-dioxide in small capitals, carbohydrates and other compounds of carbon, hydrogen and oxygen in large italic capitals, inorganic nitrogen (nitrogen, nitric acid, ammonia) in small italic capitals, organic nitrogen in black type. The path of circulation of these materials is indicated by the arrows at the ends of the lines.

Bibliography to Lecture XIX.

Aeby. 1896. Versuchsstationen, 46, 409.
Artari. 1899. Bullet. des naturalistes de Moscou, No. 1.
De Bary. 1879. Die Erscheinung der Symbiose. Strassburg.
Beijerinck. 1888. Bot. Ztg. 46, 725.
Beijerinck. 1890. Ibid. 48, 725.
Beijerinck. 1901. Centrbl. Bakt. II, 7, 561.
Berthelot. 1892. Compt. rend. 115, 569 and elsewhere.
Boussingault. 1860. Agronomie, 1, 65.
Brefeld. 1902. Centrbl. Bakt. II, 8, 24.
Bunge. 1889. Lehrbuch d. physiol. und pathol. Chemie, 2nd ed. p. 21. Leipzig.
Fischer, Alfr. 1903. Vorlesungen über Bakterien, 2nd ed. p. 163. Jena.
Frank. 1887–88. Ber. d. bot. Gesell. 5, 395 ; 6, 248.
Frank. 1888. Landw. Jahrb. 17, 441. (Die Ernährung der Pflanzen mit Stickstoff. Berlin.)
Frank. 1890. Landw. Jahrb. 19, 523. (Die Pilzsymbiose der Leguminosen. Berlin.)
Hellriegel and Wilfarth. 1888. Unters. über die Stickstoffnahrung der Gramineen u. Leguminosen (Beil. Zeit. d. Vereins für die Rübenzuckerindustrie). Berlin.
Hiltner. 1896. Versuchsstationen, 46, 153.
Hiltner. 1899. Centrbl. Bakt. II, 5, 831.
Hiltner. 1900. Ibid. II, 6, 273.
[Hiltner. 1904. Lafar's Handb. d. tech. Mykologie, 3, 24.]
Jacobitz. 1901. Centrbl. Bakt. II, 7, 783.
Jensen, H. 1898–9. Centrbl. Bakt. II, 4, 401 ; 5, 716.
[Jensen, H. 1904. Lafar's Handb. d. tech. Mykologie, 3, 182.]
Kamienski. 1881. Bot. Ztg. 39, 457.
[Keutner. 1904. Wiss. Meeresunters. Bd. 8.]
[Koch. 1904. Lafar's Handb. d. tech. Mykologie, 3, 1.]
Kossowitsch. 1894. Bot. Ztg. 52, 97.
Kühn, J. 1901. Fühling's Landw. Zeit. 1, 2 ; reviewed in Centrbl. Bakt. II, 7, 601.
Lemmermann. 1901. Kritische Studien der Denitrifikationsvorgänge. Jena.
Maassen, A. 1901. Zersetzung d. Nitrate u. Nitrite durch Bakterien (Arb. Kais. Ges.-Amt), 18, 1.
Magnus, W. 1900. Jahrb. f. wiss. Bot. 35, 205.
Mazé. 1897. Annales de l'Institut Pasteur, 11, 44 ; 12, 1.
Möller. 1902. Reviewed in Bot. Ztg. 60, II, Abt. 201. [Comp. also Bot. Ztg. 1903, II, 329.]
Nobbe. 1899. Versuchsstationen, 51, 241.
Nobbe and Hiltner. 1899 a. Versuchsstationen, 51, 241.
Nobbe and Hiltner. 1899 b. Ibid. 52, 455.
Pfeiffer and Franke. 1897. Versuchsstationen, 48, 455.
Prazmowski. 1890–91. Landw. Versuchsstationen, 37, 161 ; 38, 5.
Puriewitsch. 1895. Ber. d. bot. Gesell. 13, 342.
Saida. 1901. Ber. d. bot. Gesell. 19, 107.
Schlicht. 1889. Landwirtschaftl. Jahrbücher, 18, 477.
Schlössing and Laurent. 1890. Compt. rend. Paris, III, 750; comp. also Annales de l'Institut Pasteur, 6, 65 ; 6, 824 (Koch, Jahresbericht, 1890–2).
Schulz-Lupitz. 1881. Landw. Jahrb. 10, 777.
Shibata. 1902. Jahrb. f. wiss. Bot. 37, 643.
Stahl. 1900. Jahrb. f. wiss. Bot. 34, 539.
Stutzer. 1901. Centrbl. Bakt. II, 7, 897.
[Ternetz. 1904. Ber. d. bot. Gesell. 22, 267.]
Ward. 1899. Annals of Botany, 13, 52.
Winogradsky. 1895. Archives des Sc. biologiques. St. Petersburg, 3.
Winogradsky. 1902. Centrbl. Bakt. II, 9, 43.


Assimilation
Respiration
Sulphur-Bacteria
CARBON-DIOXIDE + ? Proteid
SULPHURETTED HYDROGEN + OXYGEN SULPHURIC ACID
Assimilation
Respiration
Nitro-Bacteria
CARBON-DIOXIDE + ? Proteid
AMMONIA + OXYGEN NITRIC ACID
Putrefaction
Proteid AMMONIA
Urea-fermentation by Uro-Bacteria
Urea AMMONIA
Proteid decomposition by animals
Proteid Urea
Nutrition of animals and heterotrophic peptone-plants
CARBOHYDRATE + PEPTONE Proteid
Nitrogen-assimilation by heterotrophic ammonia-plants
CARBOHYDRATE + AMMONIA Proteid
Nitrogen-assimilation by autotrophic nitrate-plants
CARBOHYDRATE + NITRIC ACID Proteid
Carbon-assimilation by autotrophic green plants
CARBON-DIOXIDE CARBOHYDRATE + O
Respiration of plants and animals
CARBOHYDRATE + OXYGEN CARBON-DIOXIDE
Respiration of succulent plants and Fungi
CARBOHYDRATE + OXYGEN ORGANIC ACIDS
ORGANIC ACIDS + OXYGEN CARBON-DIOXIDE
Alcoholic fermentation of yeast
CARBOHYDRATE CARBON-DIOXIDE + ALCOHOL
Acetic acid fermentation of acetic acid Bacteria
ALCOHOL + OXYGEN ACETIC ACID
Respiration of Mycoderma
ACETIC ACID + OXYGEN CARBON-DIOXIDE
Butyric acid fermentation of Bacteria
CARBOHYDRATE BUTYRIC ACID
Respiration or fermentation of Fungi and Bacteria
BUTYRIC ACID + or − OXYGEN CARBON-DIOXIDE
Denitrification
CARBOHYDRATE + NITRIC ACID NITROGEN
Fermentation
Nitrogen-assimilation
Clostridium pasteurianum
CARBOHYDRATE BUTYRIC ACID
CARBOHYDRATE + NITROGEN Proteid
Nitrogen-assimilation by Bacterium radicicola
CARBOHYDRATE + NITROGEN Proteid
or N

PART II. METAMORPHOSIS

LECTURE XX

INTRODUCTION

In the discussion of metabolism mention has frequently been made of the work done by the organism as a result of respiratory and other analogous katabolic processes. These activities were also touched on in Part I, for it must be clearly understood that the movements of raw food material, plasta, and products of assimilation are just as much expressions of such activities as the movements of an entire organ, as, for example, the assumption of the erect position by a stem laid horizontally. Having now considered chemical physiology we must turn our attention more especially to the *activities* of the organism, but in doing so it should be noted that we are changing not the subject-matter but the point of view, since chemical changes are never absent when the shape or position of a plant or plant-organ is altered. Change in material, in energy, or in form, occur simultaneously in nature, and it is only for convenience of investigation or exposition that we may legitimately institute subdivisions in our science. Two such divisions, generally recognized, are metabolism and transformation of energy; we venture to add a third, viz. change of form. Before attempting to justify the institution of this section it may be well to inquire on what the characteristic form of an organism, and especially of a plant, really depends, and also what manner of alterations it undergoes.

Let us approach the subject inductively and consider a few examples. First of all take the Myxomycetes, which we have already learned (Lecture I) to regard as naked protoplasmic masses, or plasmodia. Plasmodia are soft, slimy bodies which form irregular networks over their substrata, i. e. decaying leaves, dead branches, &c., and in which continual changes in outline may be observed with the naked eye, simultaneously with changes in position. These movements are even more apparent under the microscope, and one may observe a complete alteration in shape in the course of a few minutes. Phenomena such as these are not, however, what we mean by 'change of form', and should not be included in the present section of our studies. Just as a viscous liquid spreads irregularly over its substratum, so the plasmodium has no *definite* shape; only when it assumes such a shape does it come under consideration in this relation. Under certain conditions, however, the protoplasm of a Myxomycete aggregates, takes on a rounded form, secretes a firm external layer, and its contents divide into a large number of spherical cells. Each of these is capable under suitable external conditions of again becoming a naked mass of protoplasm and of creeping about over the substratum. The slime fungi may thus exist in two conditions, different in shape, in the vital phenomena they exhibit and in their significance. In the 'formless' state it requires a certain amount of water, inorganic and organic nutrients, and a certain temperature, and given these conditions the food-materials are assimilated and the plasmodium grows.

In the second state no vital phenomena are manifested, neither growth nor absorption of food ; though completely desiccated, the protoplasm retains its capacity for forming a plasmodium for months or even for years. We may regard the plasmodium as the vegetative, the other we term the reproductive stage of the fungus, or we may speak of it more definitely as the ' sporangium ' and describe the cells within it as spores. These spores preserve the characteristics of the slime fungus at a time which is unfavourable to vegetative life—they propagate the organism. The questions we have to ask and, as far as possible, to answer are :—What are the factors which induce the appearance of the vegetative and what of the reproductive state ? What significance lies in the fact that the former state is destitute of definite shape and that the latter has a constant shape ?

As our second example we will select a fungus, found in the frog's excreta, and belonging to the Entomophthoreae, viz. *Basidiobolus ranarum*. It is a heterotrophic plant, requiring organic as well as inorganic nutritive materials. For this fungus, as for many others, peptone and sugar form an excellent culture medium ; peptone alone, however, will serve quite well. The usual form of a *Basidiobolus* is a cylindrical cell many times longer than broad. The protoplasm is never naked but is always surrounded by a cell-wall, and in each cell we find a nucleus lying in the protoplasm, accompanied by one or more vacuoles.

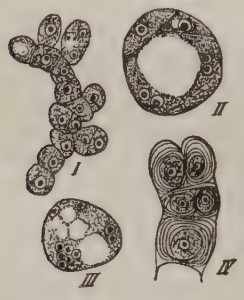


Fig. 46. *Basidiobolus ranarum*. After RACIBORSKI (1896). *I*, Grown in 20 per cent. solution of glucose. *II*, Cultivated in a 10 per cent. solution of sugar at a high temperature. *III*, Giant cell from a similar culture, without cell-walls but with several nuclei. *IV*, Palmella stage grown in a solution of glucose and ammonium sulphate.

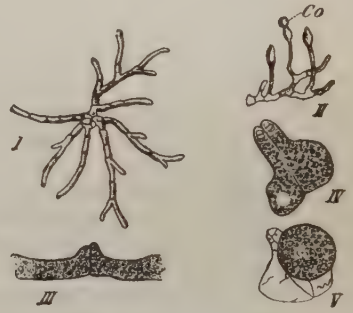


Fig. 47. *Basidiobolus ranarum*. After EIDAM. *I*, Young branched plant (× 60). *II*, Young plant with erect conidiophores. *Co*, Conidia (× 30). *III*, Early stage in the formation of a zygote (× 250). *IV*, Formation of a zygote from two conidia (× 250). *V*, Later stage of *IV* (× 250).

The cell *grows* in the culture fluid, it becomes longer, and finally forms medianly a transverse wall by which it becomes subdivided into two cells. Previously division occurs in the nucleus, and each daughter-nucleus finds its way into one of the ' daughter-cells'. The division is a concomitant of growth, and although both cells remain united, each possesses an individual vitality, and exhibits all the features of a *Basidiobolus*. The cells may be isolated and each cell is then seen to be quite independent of the other, growing and dividing on its own account. Growth then takes place not in one direction only, the long axis, but lateral branches appear (Fig. 47, *I*) which, however, do not call for further mention here. If the composition of the culture remains unaltered growth and cell division proceed for an unlimited time in the same way. On the other hand, remarkable changes in form may be induced by altering the composition of the nutritive solution (RACIBORSKI, 1896). If the *concentration* be greatly increased and a 20 per cent. solution of sugar be supplied instead of 10 per cent., or if sodium chloride or another mineral be added up to 6 or 10 per cent., growth in length is inhibited, the cells become more rounded, and the

division walls are no longer strictly transverse but oblique (Fig. 46, *I*). Finally, especially under high temperatures, cell division ceases entirely, though growth and nuclear division continue and giant cells with many nuclei result (Fig. 46, *II*, *III*). We have now obtained forms which are no longer typical, since they have lost their power of normal development and cannot regain their original shape. Shapes not less remarkable, but still normal, may be obtained by a *qualitative* alteration of the nutritive solution, viz. by retaining sugar as the source of carbon but by employing ammonia or some related body (amines) in place of peptone. The cells now become more rounded and divide irregularly in all directions; further, the walls surrounding the daughter-cells become stratified in a remarkable manner (Fig. 46, *IV*). Each cell is surrounded not only by its own cell-wall but by that of the mother-cell, and finally by that of the parent cell of all. The membranes then gradually degenerate and the cells become free, separate from each other, and take on a spherical form. This growth form reminds one of certain lower Algae and may, as in that case, be termed the ' palmella ' form. By keeping the nutritive solution constant, *Basidiobolus* may be made to continue growing in the palmella form for an indefinite length of time.

Since every cell is physiologically independent, multiplication results from every division; as is the case with many of the lower organisms, it is impossible to separate vegetation from reproduction. *Basidiobolus*, however, also forms special reproductive cells, and these have *one* function only—they are not at the same time vegetative.

Conditions are complicated by the fact that two kinds of reproductive cells are produced; the one type is the so-called conidium (Fig. 47, *II*), which arises in the following way. A swelling appears at the end of one of the cells which stand up erect out of the nutritive substratum; into this swelling almost all the protoplasm, together with the nucleus, migrates. The swelling is finally segmented off by a transverse wall and the conidium is complete; it is afterwards thrown off by a special mechanism. The function of the conidium is not to *increase the extent* of fungus in the same substratum, but to *distribute* it to another. The other type of reproductive organ—the zygospore—arises in quite a different way. Two cells of a filament develop beak-like projections at the limiting wall (Fig. 47, *III*), which in that situation become dissolved, so that the protoplasm of one cell, or at least most of it, can wander over into the cavity of the other (*IV*, *V*). The protoplasts thus fuse and the product becomes enclosed in a very thick wall, which is always characteristic of this type of spore, and indicates that the cells concerned are able to, or must, pass through a long resting period. The two nuclei of the zygospore fuse later. Zygospore formation is a sexual process of a very simple type, the spore being formed by the fusion of a male (the motile cell) with a female cell.

Thus *Basidiobolus* has both sexual and asexual reproductive organs, and these are distinguished not only by their mode of origin but also by the *conditions* under which they are formed and by their *significance* in the plant's life-history. It has already been noted that the conidia are formed outside the medium; the zygospores, on the contrary, are *only* developed in it. Both structures, however, appear only when the nutritive solution begins to become exhausted. The formation of either type of reproductive organ may be suppressed by periodical renewal of the culture fluid.

Basidiobolus is thus an organism showing very varied shape and modes of development, and the variations observed in it demand our special attention, depending as they do in the most obvious manner on external conditions which are determinable at will. This example teaches us how external factors may influence the shape which the plant assumes.

It is unnecessary for us to describe all the intermediate stages in plant shapes that exist between a simple fungus and the most highly developed plant,

so that we may pass from *Basidiobolus* directly to our third and last example—the *flowering plant.* In its earliest stage the flowering plant is nothing more than a single cell (the ovum), similar in all respects to the cell of a fungus. In this case also the cell grows and divides, but all the daughter-cells do not fulfil the same function nor do they continue to have the same structure ; they are no longer of equal value and must remain connected if they are to continue in existence. It is not the *cells* of which the higher plant is composed that attract our attention in the first instance but rather the *external* members of the plant ; the cell structure of each individual part is revealed on subsequent microscopical investigation. The external segmentation of the plant is, however, already suggested in the embryo whilst still within the seed, exhibiting as it does two poles, one of which we term the radicle, the other the plumule. The distinction between them exhibits itself most clearly during germination by the fact that the root on leaving the seed pushes its way downwards into the soil while the plumule grows in the opposite direction. A careful study of the conditions under which the differentiation of these two poles arises forces us to the conclusion that we must seek its cause *not* in *external* factors, such as play so important a part in the morphology of *Basidiobolus*, but in some *internal* factors. The further progress of development in a flowering plant also convinces us that internal causes play a most important part, for though the external factors remain completely unchanged the plant itself alters in shape from day to day. The root grows apically and increases in length in a well-marked manner ; it forms lateral branches which resemble the principal root in all essential respects. The plumule divides into axis and leaves and gives origin to lateral axes, also provided with leaves. The leaves may be of diverse shape, but usually agree in exhibiting vigorous unilateral growth, forming thin but at the same time broad surfaces, green in colour, owing to the chlorophyll contained in their cells. They change their shape several times in the course of the vegetative period ; the typical foliage leaves are preceded by simple leaf-like organs, and are followed by them also ; the branch produces alternately scale and foliage leaves, or at the apex proceeds to form bracts, and finally flowers. In the flowers are developed the organs of sexual reproduction, by whose agency new plants are formed. In addition to sexual organs we find in thousands of flowering plants organs of vegetative reproduction as well.

The flowering plant passes through a ' life cycle ' which, at least in certain cases, begins with the germination of the seed and ends with its formation, and which extends over one or more seasons. We found that in *Basidiobolus* the various growth forms were mainly dependent on external conditions, but in the flowering plant we have something entirely different, namely the intimate connexion between the functions of the various members and their external and internal structure. We have seen, in Part I, how all the parts of the higher plant no longer fulfil the same functions, as they do in unicellular forms. The root, we have found, is the organ for absorption of water and soluble salts from the soil, the roothairs being specially concerned in this absorption. There can be no doubt that these roothairs, by vastly increasing the surface of the root, by their intimate union with the soil particles, and by their excretion of certain substances, are specially adapted for this purpose. The substances acquired in this way are, in part, transferred to or elaborated in the aerial parts of the plant, and hence we find special conducting strands developed in the interior of the root which are in intimate connexion with similar conducting channels in the shoot. The chief of these are the tracheae, cells which have lost their protoplasmic contents and consist of empty tubes into which the water flows.

The function of the leaf is entirely different. It is the carrier of the chlorophyll by means of which carbohydrates are formed from carbon-dioxide. For this purpose, as we have already seen, sunlight is essential. The chlorophyll has

thus to be exposed to the sunlight in a thin layer, and we may regard the flattened form of the leaf as specially adapted to fulfil this function. SACHS (1882, Vorlesungen über Pflanzenphysiologie, p. 618) has shown in a most interesting manner how the essential structural relationships of the higher plants are subservient to this chlorophyll-function. We can thus understand how the leaf must have an entirely different shape from the root. The greater its surface the greater the transpiration, and consequently the arrangement of the water-conducting tissue in the leaf is especially adapted to carry out this function. In order, however, that transpiration may not be so great as to act inimically to the plant, we find numerous arrangements (to which we have already referred) for retarding that process.

If we turn now to the stem we find it to be the medium of communication between the root and leaf; it has to carry the substances absorbed by the root up to the leaf and, conversely, to transport back again the materials manufactured in the leaf. It has also to support the entire weight of the aerial part of the plant, its own as well as that of the lateral organs; and when we consider, not a small annual herbaceous plant, e. g. *Draba verna*, but an oak-tree several hundred years old, we can easily appreciate how rigid the stem must be. Each cell by itself has a certain rigidity owing to the tension of its membrane, due to the osmotic activity of the cell-sap. This alters, however, with the amount of water present, and on hot summer days the rigidity due to turgescence is rapidly reduced by increased transpiration. All land plants of large size have, therefore, as well a *special* mechanical tissue system, the thick-walled sclerenchyma. We owe to SCHWENDENER (1879) the demonstration of the fact that this sclerotic tissue is arranged in accordance with engineering principles, so that the greatest effect is attained with the minimum expenditure of material. Sclerotic tissue is also present in the leaves and roots, but it is distributed differently in these situations, since the mechanical requirements of these organs differ from those of stems.

In this sketch we have limited ourselves to the vegetative organs and have referred only to some of the most characteristic features exhibited by these organs. A detailed description of the anatomical adaptations seen in plants will be found in HABERLANDT'S (1896) Physiologische Pflanzenanatomie, 2nd ed., Leipzig; to give such an exposition here would be foreign to the purpose of these lectures. The general result, however, of our review is to lead us to the conclusion that the structure of a member is *adapted* completely to the function it has to perform. Doubtless, also, the cell of *Basidiobolus* is adapted to the performance of its functions, although on account of the fact that all functions are in that case carried out by *one* cell, the finer organs and their adaptations are microscopic and cannot be distinguished in detail. It is not to be wondered at that we do not know whether an elaborate *differentiation*, such as occurs in the body of the higher plant, or such a purposeful *division of labour*, occurs here also, or whether the single cell taken as a whole is able to act like the complex apparatus of a higher plant, made up of millions of cells.

Division of labour in differentiated plants has a far-reaching significance, on which a few remarks must be made here. Whether the two cells resulting from the division of the original *Basidiobolus* remains united or not is quite immaterial, but it is quite a different matter in the case of the flowering plant. Here the individual parts, whether they be the members visible to the naked eye or the cells seen only under the microscope, are incapable of living *separately*. A leaf, for example, torn off by a gale of wind rapidly dies; it can manufacture organic substance, it is true, but it withers for want of water. A root also can take water and salts from the soil after the shoot has been cut off, but it soon ceases to grow, because no organic materials are supplied to it. An isolated sclerenchymatous fibre or trachea removed from the organism is a dead and useless structure. Only when these units are bound together into a concrete

whole are they capable of performing their functions adequately, and only then is it possible for the whole structure to grow and thrive. In this way we can see that *correlations* become established among the various parts, one essential result of division of labour. These correlations have, however, a much greater influence on the general shape of the plant than we might conclude from these remarks.

Numberless correlations make their appearance if members be *isolated*, if leaves, branches, or roots of plants be separated off and prevented from rapid decay by appropriate artificial means. The capacity for *regeneration* then makes itself apparent, that is to say, the capacity of a member to reconstruct the whole body by budding out the missing organs. The root can give rise to shoots, the shoot to roots, the leaf to both shoot and root. The normal plant gives us no hint of this power, and yet that power must have been latent in it; the inter-relationships of the members only must have prevented the individual organs from exhibiting all the capacities which they possess. Owing to this correlation the growth and formative power of the different members are *regulated* and made subservient to the whole in such a way that the structural evolution which we are accustomed to see in the plant proceeds *harmoniously*. What is true of the root and shoot as a whole is true also of the individual cells. Numberless myriads of parenchymatous cells die off at last when they have reached a certain stage in development and after they have lived a long time in that stage. They can all be induced to form every possible kind of cell by inhibiting correlations, and hence may be made to continue alive for indefinite periods. If this subordination of cells did not exist in the multicellular plant each cell would endeavour to develop as much as it could, and then we should have not an organism but such a chaos run wild as to make existence impossible.

The flowering plants are perpetually altering their shape, and the organs to which they give rise are not only so far adapted that they perform the specific functions which enables them to carry out their structure, but also in so far that they do not exhibit all the activities of which they are capable. The entire life-cycle of the plant from the germination of the seed to the formation of seed takes place under constant external conditions, so that we are unable to refer these changes in shape to these factors with the same degree of accuracy that we did in the case of *Basidiobolus*. Nevertheless, this life-cycle is affected by external influences, and that, too, in a double sense.

Whenever a seed gives rise to a seedling the first thing it requires is a certain amount of *water*; that is self-evident, since we have seen that water is an absolutely essential constituent of the living organism. Since the seed in a state of rest is quite air-dry, the addition of water is necessary to awaken its activities. If a member of a plant has accumulated a store of water during its resting period it is able to start developing without any such addition. But not only must water be absorbed, it must also be immediately *available* if growth is to be effected, and the same is true for all other substances needed for this purpose. They must be absorbed, or have been absorbed previously, and in that respect every growth phenomenon in a plant is dependent on the external world; this, however, requires no further elucidation—it is self-evident. The influence of *temperature* on development is not so obvious. Yet a glance at re-awakening nature in spring time indicates to us what part temperature plays in vegetative phenomena. Experiments confirm this and show that the carrying out of every individual function in the plant is dependent on the existence of a certain amount of heat. Beans first show evidence of growth when the temperature reached 9° C.; growth increases as the temperature rises (up to 34° C.), and finally ceases when 46° C. is reached. Three cardinal points of temperature, a minimum, optimum, and maximum, may be established for all organisms, and the very diverse positions of these cardinal points indicate the varied requirements of organisms as regards temperature, and at the same

time demonstrate the great importance of this factor in determining the geographical distribution of the plant. Since certain Algae have their optimum about 0° C., and certain Bacteria at from 60° to 70°, it is manifest that they must necessarily be inhabitants of very different regions. This example shows us that external factors are indispensable conditions of plant development, and that, too, not in the case of the higher plants only but in the case of all organisms. We term these 'formal' conditions of development to distinguish them from special formative external influences which on closer examination affect the higher plants also.

On examining plants of different families which live together under one or other extreme, we find that they have one capacity in common, viz. that they can adapt themselves to these extremes (compare VOLKENS, 1887, GOEBEL, 1889–93, 1, 25; 1, 149; 2, 3; KERNER, 1891, &c.). Thus desert plants, which have difficulty in satisfying their requirements so far as water is concerned, exhibit numerous adaptations for retarding transpiration; their surface is limited in extent by reduction in the size of the leaves, the function of assimilation being undertaken by the stem (Cactaceae and Euphorbiaceae); further their cuticle is thickened, their stomata are deeply seated and they cover themselves with layers of wax or hairs. On the other hand, we find in them many arrangements for bringing about a maximum absorption of water if such be available; they exhibit an especially extensive and deeply penetrating root-system.

In marked contrast to these 'xerophytes' are the 'hydrophytes' or aquatic plants, especially the submerged types which we shall now consider (compare ASKENASY, 1870; SCHENCK, 1886; GOEBEL, 1893, 2, 215). These forms are capable of absorbing water by their entire surface and have nothing to fear from loss by transpiration. Accordingly, the root in these plants is quite in abeyance as a water-absorbing organ; the water-conducting tissue is feebly developed; the cuticle is thin and easily permeable and the mechanical tissue is often entirely wanting. Submerged plants experience, however, difficulties in gaseous exchange. They can obtain gases only from the surrounding water, and thus, doubtless, we may account for the enormously increased leaf-surface produced by the formation of numerous delicate projections. Roots and rhizomes especially which are imbedded in mud must be supplied with the necessary oxygen from the parts above, and hence may be explained the extraordinary development of intercellular spaces which is a feature of all hydrophytes. Stomata, on the other hand, the normal apertures for gaseous exchange in land plants, are wanting entirely in submerged forms.

These brief notes may suffice to show that plants *adapt* themselves to their surroundings. We should be incorrect, however, in assuming that the special shape of the hydrophyte was in any sense *induced* by the medium in which it lives. We are acquainted with forms, the so-called amphibious plants, which are capable of living both on the land and in the water. We may note especially, in illustration, *Polygonum amphibium*, whose land and water forms differ remarkably from each other. When in water, the rhizome is long and obliquely ascending, bearing several leaves with long petioles and heart-shaped, broadly lanceolate, leathery blades floating on the water. The entire plant is smooth and glabrous. When grown on land the stem is erect, the leaves are narrow lanceolate, *quite sessile*, wrinkled, and partly hairy. *The aquatic and terrestrial forms may exist, however, concurrently as two branches of the same rhizome.* The aquatic form of *Ranunculus aquatilis* possesses extremely finely divided leaves and long internodes, the land form has short internodes and broader leaf apices. The anatomical differences between these leaves are especially striking; those of the land form are rigid, bear stomata, and have their assimilatory tissue dorsiventrally arranged; while those of the aquatic type are soft, have no stomata, and have radially-arranged assimilatory tissue. Still in this plant also *it is possible by cultivating the terrestrial form in water to turn it into the aquatic type.*

Since it has been shown that here water itself has a formative influence on the plant and induces adaptations directly, we are bound to conclude that in other cases, as in aquatics which no longer have land forms, and in xerophytes which do not alter their habit although in the presence of abundant water, the direct effect of the medium does not exhibit itself in the life-cycle of the *individual*, but has developed during the evolution of the *species* and has now become permanent. We involuntarily reach the conclusion that species are variable, but that many of their characteristics are *hereditary* adaptations.

Although the formative effect of the external world has been abundantly proved we must not suppose that the plant reacts to all factors with purposeful adaptations. We need advance only one example of such a reaction due to external influences which does not appear to be of any service to the plant, namely, the palmella form in *Basidiobolus*. It is often by no means easy to determine whether a change in shape is to be considered as an adaptation or as ' a product of a fortuitive mechanism ' (BERTHOLD, 1898) ; for, in keeping with the views they hold on certain general questions, some botanists are inclined to look for adaptations everywhere and to find them, whilst others are content to discover merely the operation of a purposeless mechanism. In the latter case they follow in the footsteps of physicists and chemists, and recognize in the organism the selfsame forces which operate in the inorganic world. If we look on all these changes as ' adaptations ', we have still the all-important problem to solve: why does the plant react in a purposeful way? It reminds one of an organism possessed of intelligence, and the problem appears to be beyond our power to solve.

We come now to a most important question, viz. whether results springing from the operation of these forces in the organic world obey the same laws which they do in the inorganic, or whether we have here to deal with quite special relations. Before attempting to discriminate between these two alternatives let us glance at what we have learnt in this connexion from our experimental treatment of the problem of plant formation. Wherever we look we are forced to the conclusion that every change in an organism is a complex process, which is due never to one solitary cause but to a large number of co-operating factors. The phenomena are thus remarkably complicated, and when we compare them with those of other sciences the probability of being able to arrive at a mathematical-physical explanation of them is very slight. As every one knows, Astronomy can calculate with the greatest exactness the path by which a body in obedience to the law of gravity moves towards another ; if a third body makes its appearance influencing the path of the first, the course may still be determined *empirically*, though no longer strictly *mathematically*. Glancing now at meteorological phenomena no one doubts that they obey simple physical laws ; in principle, these are quite intelligible, but an explanation of an individual case or an exact prediction of a meteorological condition is not possible. If then in any science, *only* that may be considered as *explained* which can be expressed in terms of *mechanics*, how may we dare to hope ever to arrive at a physical explanation of life ? Still, as in the science of Meteorology, we may succeed in reaching, at least, a knowledge of *principles*. In inanimate nature alone there are plenty of phenomena which mock our attempts to refer them to mechanical causes, e. g. the inherent characters of bodies. The peculiar *characteristics* of an *element* are incomprehensible and inexplicable ; even more inexplicable is the fact that compounds of these elements assume new characters not to be referred to combinations of those of the elements themselves. It is impossible to affirm that the characteristics of living bodies are distinct in *principle* from those of non-living ; all we can say is that we are equally debarred from a knowledge of those of either. Generally speaking, a *mechanical* explanation of life is out of the question ; at most a *physico-chemical* explanation is all we can hope for (ALBRECHT, 1901).

Many of the phenomena which we have become acquainted with suggest comparisons not only between organisms and complex conditions in the non-living world, but also in another direction. We can distinguish *internal* and *external* causes in vegetable phenomena; only when these work in harmony is development or any other activity possible (C. BERNARD, 1878). Take the bean, for example. Germination takes place only if certain *external* conditions be fulfilled; there must be certain materials present in the medium in which the development takes place, and also water and oxygen; again, there must be a certain temperature, and in the later stages, at least, sufficient illumination. That co-operation of *internal* factors on the other hand is essential is shown by the fact that identical external conditions can induce no development in seeds which have died after prolonged keeping, but which are otherwise unaltered, and further that bean plants always arise from bean seeds, while from peas an entirely different type of plant arises. It would be an arbitrary proceeding to assume that any one of these many causes is the *chief factor* in the phenomenon concerned.

The activity of a piece of machinery is also dependent on the interaction of internal and external factors. Its specific activity depends on the arrangement of its constituent parts, and it is only when these parts are co-ordinated in a systematic manner that it can perform its functions properly. But if the machine is to do work the external conditions must also be fulfilled—in a steam-engine, for example, when a certain pressure of steam acts on the piston. Hence it is frequently the custom to compare an organism with a piece of mechanism, and this comparison may be carried further when the significance of each individual factor in the performance of work is taken into account. In the plant, as in the machine, we may distinguish certain factors which directly provide it with *energy* to carry out the work, and others which may be looked upon as merely *liberating* energies. The opening of the stop-cock which permits the steam to enter the piston-box is a liberator of this type; so is the pulling of the trigger of a rifle. In neither case does the necessary pressure of the finger bear any direct relation to the work done by the machine; it only releases a pre-existing energy and allows it to perform work. The work in the one case is done by the expansion of steam, in the other, in the first instance, by the spring of the rifle, and then by the explosive force of the powder. In the plant only a few cases are known in which an *external* factor supplies *directly* the energy required for the production of the result, e. g. the action of sunlight in carbon assimilation, or the sugar in the nourishment of heterotrophic plants; in the large majority of cases the external factors merely liberate energy, that is to say, act as 'stimuli' (PFEFFER, 1893)—and the work is done by energies already stored in the plant. It is very frequently the case in the plant that one released movement releases another and so on, so that a whole series of reactions may occur between the obvious primary release and the obvious final result, just as in the case of the rifle between the pulling of the trigger and the impact of the bullet on the target. The plant is, in a sense, 'loaded' and ready to transform its potential energy into kinetic whenever the necessary stimulus is applied.

Another important similarity between an organism and a machine lies in its power of self-regulation. Just as in a steam-engine an excessive speed is reduced *automatically* by that very increase, so in an organism similar self-regulating mechanisms occur; compare in this relation what has been said as to the production of diastase (p. 183).

Differences between organisms and machines are, however, not wanting. We must take into account, in the first place, the much more *complicated* nature of the organism, in which respect naturally there is no fundamental difficulty in making a comparison. Although we have compared the organism with a loaded rifle, still we must note that this comparison gives us but a feeble idea of the

mode of life of an organism. We have only *one* act of releasing and *one* reaction in a rifle, whilst in an organism we have numberless liberations of energy and all sorts of reactions. We have a further more important difference to note, viz. that one of the chief activities of an organic machine lies in its special *structure*, its *development* and its *reproduction*, while we have still to find a machine which can grow and reproduce. Finally, we know that the machine works to achieve a certain end, being constructed for that very purpose, but we cannot do more than form the vaguest guesses as to the purpose for which the organism works.

To sum up ; the causes of life are as yet *entirely unsolved*, we are ignorant both as to the materials and the forces which give to living things their characteristics ; just as little are we able to prove that *other materials* and *other forces* operate in the organisms than in the non-living world. Our position with regard to biological research may be permitted to rest with this expression of our ignorance, since the enunciation of hypotheses on questions so general as these would only too easily do injury to *Natural Science*. He who believes that the organic world is nothing more than a collection of complicated chemical and physical processes can only do so by shutting his eyes to such phenomena as cannot be fitted into his theory ; he who, on the contrary, once admits that the vital characteristics of the organism begin where physics and chemistry end, is content to abandon altogether the toilsome path of exact investigation, and aim merely at collecting the most readily accessible results of speculation at his study table. As to the possibility of reaching an explanation of vital phenomena the following works dealing with the subject may be consulted :— Albrecht (1901), Bütschli (1901), Claussen (1901), Driesch (1901, [1905]), Hertwig (1897–8 [and 1905]), Nägeli (1860), Reinke (1901), Wolff (1902). [There can be no doubt, however, that physico-chemical experimental investigation and not philosophical speculation has been the chief means of advancing the science of plant physiology.]

From the examples considered above we can readily appreciate the kind of questions we have to study in the lectures yet to follow. In the present lecture we need only attempt to justify the allotment of a complete section of physiology to the discussion of the 'form' of the plant and to show that this question of 'form' may be contrasted in a certain sense with 'material' and with 'energy'. If we study the introduction to Sachs's famous memoir, 'Ueber Stoff und Form' (1880), it would appear as though this statement were to a certain extent subject to criticism. Sachs says, 'Plant morphology often suffers the misfortune of being considered from the point of view of form without any reference to its material characteristics'. 'A survey of the material characteristics of organs' is, however, absolutely necessary '*since it is in these only that the causes of their form are to be sought for*'. 'Just as the form of a drop of water or of a crystal is the result of the action of certain forces which bring the material in question under the influence of its surroundings, so also *the organic form can only be the outward result of forces which transport those materials which make themselves apparent in the plant substance.*'

Valuable as are the opinions which Sachs has expressed in this treatise on the subject of 'causal morphology', we are nevertheless unable to agree entirely with the sentiments expressed in the sentences quoted. We cannot find that Sachs, or indeed any other author, has succeeded in referring the form of an organ to its material characteristics, and, keeping before our eyes the phenomena of non-living nature, we must confess that it is not probable that anything of this kind is ever likely to be established. Numerous chemical compounds have characteristic crystalline forms, and often these forms serve for the diagnosis of different bodies. Still the same form may be possessed by different materials and it would be in the highest degree dangerous to refer the leaf form, for example, to one special but as yet unknown material, and still more dangerous

to attribute the various types of form seen in leaves to differences in these materials. But even if that were possible, we must still, as in mineralogy, treat of the *form* of a plant by itself ; and even though we may be able to prove that a definite form results from the presence of a definite material constitution still we do not know *why* it is so ; why, for example, calcium oxalate crystallizes in a tetragonal form when three molecules of water are present, and in the monosymmetrical form when there is only one. Nowadays, when an explanation of form as due to chemical constitution is still quite impossible, it appears to us that a special section dealing with ' metamorphosis ' is essential.

It is, perhaps, not possible to sharply separate off change of form either from the second department of physiology, the transformation of energy —more especially the phenomena of movement—or from morphology. However, the delimitation is of service from the practical point of view, since a large series of vital phenomena, especially those connected with reproduction, heredity, evolution of species, &c., may be most naturally classed along with other formative processes, while they cannot be treated of in a binary classification of the subject nor be dealt with in an appendix. This, however, is of no consequence so far as the plant is concerned.

In the following pages we have to investigate first of all how growth and formation is carried out under constant external conditions (Lectures XXI–XXIII) ; we shall thus learn to appreciate the *mode of action* of internal causes of growth, although we shall not gain thereby any closer insight into their nature ; at the same time we shall endeavour to form a conception of what growth and formation really are. Thereafter we will devote ourselves to the study of the influence of the most important external factors on growth (Lectures XXIV–XXVI). In conclusion we will devote ourselves to the study of the *developmental life-cycle itself*, which results from the simultaneous operation of both internal and external factors.

Bibliography to Lecture XX.

Albrecht. 1901 Biolog. Centrbl. 21, 97.
Askenasy. 1870. Bot. Ztg. 28, 193.
Bernard, Cl. 1878. Leçons sur les phénomènes de la vie etc. Paris.
Berthold. 1898. Unters. zur Physiologie der pflanzl. Organisation, 1. Leipzig.
Bütschli. 1901. Mechanismus u. Vitalismus. Leipzig.
Claussen, J. 1901. Jahrb. d. hamburg. wiss. Anstalten, 18.
Driesch. 1901. Die organischen Regulationen. Leipzig.
[Driesch. 1905. Der Vitalismus als Geschichte und als Lehre. Leipzig.]
Goebel. 1889–93. Pflanzenbiolog. Schild. Marburg.
Hertwig. 1897. Mechanik u. Biologie. Jena.
Hertwig. 1898. Die Zelle u. die Gewebe, Vol. 2, Chap. 5. Jena.
[Hertwig. 1906. Allg. Biologie. Jena. (Cited by Wolff, 2nd ed. 1905.)]
Kerner. 1891. Pflanzenleben. Leipzig and Vienna.
Nägeli. 1860. Beitr. z. wiss. Botanik, 2, 46.
Pfeffer. 1893. Die Reizbarkeit der Pflanzen. (Verhandl. d. Gesell. deut. Naturf. u. Aerzte.)
Raciborski. 1896. Flora, 82, 107.
Reinke. 1901. Einl. in die theoretische Biologie. Berlin.
Sachs. 1880. Stoff u. Form der Pflanzenorgane. Arb. Würzburger Instit. 3, 452. (Ges. Abh. 2, 1159.)
Schenck, H. 1886. Biologie d. Wassergewächse. Bonn.
Schwendener. 1874. Das mechanische Prinzip im anat. Bau d. Monocotylen. Leipzig.
Volkens. 1887. Flora d. ägypt.-arab. Wüste. Berlin.
Wolff, G. 1902. Mechanismus u. Vitalismus. Leipzig.

LECTURE XXI

THE GROWTH OF THE CELL

THE most simply organized plants are unicellular, and the microscope shows us also that in the most complicated forms cells and their derivatives constitute the units out of which these plants are entirely built. The cell is thus to be considered as the *fundamental constructive unit* of the plant (and incidentally it may be mentioned of the animal also). Any attempt to investigate the growth and formation of the plant will thus naturally commence with a study of *cell-structure*. We will assume that all the conditions necessary for the development of the plant have been fulfilled and that the determining external factors remain *constant*.

What do we understand by the terms 'growth' and 'formation'? It is obvious in the first place that the growing cell *increases in size*, but not every increase in size is a case of growth. If we place some seeds in water we at once observe a rapid increase in size owing to the enlargement of the individual cells; but it is due merely to the introduction of water into the organic substance, to a process which we have termed imbibition. If we place the swollen seeds in the air, the water evaporates and the seeds regain their original dimensions. If we place the cell of an alga which has been plasmolysed by cane sugar (Lecture II) in water, the cell increases in size owing to the absorption of water, but the mode of absorption in this case differs from imbibition. *Excess* of water is taken into the vacuole, but it induces no separation of the individual particles of the wall and of the protoplasm. Generally speaking, this increase in size, owing to turgor or imbibition, produces changes which are *temporary*, while in true *growth* the changes are *permanent*. 'Growth' is for the most part accompanied by an increase in volume, but there are cases in which increase in one direction is accompanied by a decrease in another. In the latter case, elongation can take place without any change in volume; still we may also speak of it as growth if the change be *permanent*.

We might speak of 'formation' equally well when the organism forms cells and when the cell has a specific shape in relation to the life-conditions. But so interpreted 'formation' would be no subject for science. The causes of cell-formation we cannot indeed ascertain because we have never seen non-cellular organisms (SACHS uses the term 'non-cellular' in quite another sense from this. 'Lectures on Plant Physiology')—only on theoretical grounds we suppose their pre-existence (or also present existence) and regard them as the simpler precursors of the cell. But if in speaking of 'formation' we think of changing of shape then we acquire a notion of some fertility. A changing of shape may happen alike in swelling and in osmotic enlargement as well as in growth. But all these processes might go on also without a changing of shape, if, for instance, the body simply became larger without any alteration in its proportions; in this case we would not speak of a change of shape.

We have already discussed the most important constituents of the cell and their interrelationships elsewhere (Lecture I). Two of these only are of interest to us at present, viz. the protoplasm and the cell-wall, and we will attempt now to deal with their growth and form. We will begin with the protoplasm, the essential living substance, and by far the most important so far as our problem is concerned. Unfortunately, our knowledge of the growth of protoplasm is very limited; we know little more than the *mere fact that protoplasm grows*. It is possible to observe in many cells the actual increase of protoplasm

under the microscope, doubling its amount in some circumstances in twenty to thirty minutes, but we are quite ignorant how the protoplasm is formed from the nutritive substances supplied to it; we can only say that we are dealing with a process of assimilation, or, more accurately, *the* process of assimilation (compare DRIESCH, 1901). What we have hitherto spoken of as 'assimilation' has been the comparatively simple synthesis of organic substances. The chloroplast synthesizes carbohydrates, but the carbohydrates are no more like the chloroplast than the carbon-dioxide is; they want *life*, an essential characteristic of the chloroplast. We can only speak of assimilation in the real sense of the term when the raw materials are transformed into a *living state*, and this is what takes place when protoplasm grows, when, in other words, *new* protoplasm is formed. The characteristic feature of the organism is, more than any other, the way in which protoplasmic growth is accomplished. When a crystal grows, it finds the same material already dissolved in the matrix; the protoplasm, on the other hand, constructs itself out of *substances unlike itself* but *always* as an addition to protoplasm there already. We are quite unable, at present, to say how the process is carried out, since we are quite ignorant what protoplasm itself really is.

The newly-formed protoplasm must in some way or other be incorporated in that already present; in a word it must be *added to* it or *interpolated between* its constituent parts. To the question 'where does the protoplasm grow?' we can give no definite answer, for it leads us at once to another as yet unsolved problem, viz. the ultimate structure of the protoplasm. The position we take up as to the various theories of protoplasmic structure will determine what view we take as to the mode of growth of protoplasm. It is needless for us to follow out the various explanations which have been given, since none of them have been generally accepted; nor do we by accepting any of them gain any deeper insight into the nature of protoplasm. What applies to protoplasm in general applies also to its members, the nucleus and the chromatophores; we see *that* they grow, but we do not know *where* nor *how*.

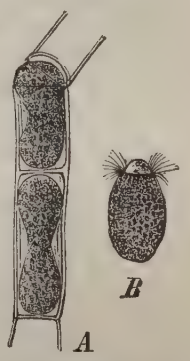


Fig. 48. *Oedogonium*. *A*, two cells whose contents are in process of transformation into swarmspores; *B*, free swarmspore (× 350). After PRINGSHEIM, from the Bonn Textbook.

We may omit any discussion of the formation of protoplasm since, for the most part, it has no definite form. It is a viscous fluid, whose form is determined frequently by external forces. It is only when its external layer has attained a firm consistence that we may speak of it as having actually morphological formation. The cases in which that condition is reached, however, lead back to others where the protoplasm has no such characteristic.

We are much better, though still imperfectly, acquainted with the mode of growth of the cell-wall. The chief difference between the protoplasm and the cell-wall cannot be more clearly expressed than by saying that formation of new protoplasm takes place only by addition to that already existing, whilst a cell-wall may be formed where no cell-wall existed previously; the formation of a cell-wall necessitates the presence of protoplasm but not of another cell-wall; the protoplasm makes itself, the cell-wall is made by the protoplasm. This dependence of the cell-wall on the protoplasm is the first thing that strikes us in a study of its earliest beginnings, and we may start with a consideration of the initiation of the cell-wall (STRASBURGER, 1898). Swarmspore formation takes place in many Algae and Fungi. In the simplest case (Fig. 48) the contents of the cell retreat from the wall, and finally escape through a crack in the wall into the surrounding water in which the free mass moves about in the water as a naked 'swarmspore'. After a certain time the movement ceases, the swarmspore

fixes itself and forms a new cell-wall, in the form of an *excretion* on the outside of the protoplasm. In other cases, also, the cell-wall arises by excretion, but this mode of formation is not universal; for it has been definitely shown that in some cases the wall arises by *transformation* of protoplasm, i. e. by a *direct alteration* of protoplasmic strands. When this method is adopted, carbohydrates must be split off from the protoplasm and the nitrogenous remainder be withdrawn, because the wall so formed exhibits the same characters as that formed by excretion. Cell-walls which arise by this solidifying of the protoplasm must have an extremely complicated composition, and CORRENS (1898) has shown that this is indeed the case. Cell-wall formation by excretion is much more common than by transformation of the protoplasm.

Cell-wall formation may also be artificially induced. A new wall may appear under certain conditions on the surface of the protoplasts of plasmolysed cells, and, further, isolated protoplasmic particles (e. g. in the Siphoneae) may be induced by mechanical means to form cell-walls.

The majority of cell-walls have, apart altogether from their mode of origin, *the power of growth;* they increase both superficially and in thickness. Surface-growth takes place first, growth in thickness follows later and continues long after surface-growth has ceased. Although these two processes in many cases

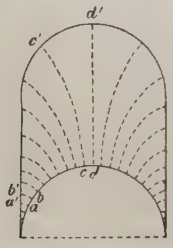


Fig. 49. Diagram of apical growth in a fungus hypha. After REINHARDT (1892).

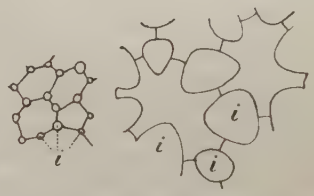


Fig. 50. Stellate parenchyma of *Thalia dealbata*; *I*, in the young state; *II*, more fully developed. After ZIMMERMANN (1893).

take place at the same time and affect each other, we will as far as possible treat of them separately. Let us begin with superficial growth, which is of importance from the point of view of the configuration of the organism, since the shape of the adult cell is determined by the superficial growth of its membrane. We have already made ourselves acquainted with the temporary alterations in form of cells produced by turgor only (stomata, p. 39), and we will recur to these changes elsewhere.

Let us endeavour, in the first place, to gain some idea of the different types of superficial growth. We know of only a few cases where superficial growth takes place *equally on all sides*, where an increase takes place without any alteration in form; as examples we may cite approximately tetrahedral pollen-grains and spores (compare Fig. 54, p. 264) and the cylindrical cells of *Hydrodictyon*. Generally, only certain parts of the cell-wall grow superficially and these parts may be distributed in various ways on or between parts which show no such growth. Examples of such *localized* surface growth are to be seen in the simple spherical cells of some Algae (Pleurococcaceae) which are hemispherical in the young state and are hence limited on one side by a flattened cell-membrane. This flat surface alone grows and transforms the hemisphere into a complete sphere. In many cylindrical cells also, e. g. in the Conjugatae, surface growth is localized, for the *cylindrical* wall alone grows while the disk-shaped transverse walls retain their original dimensions; the cell-wall increases in extent but not in thickness. In both the examples we have selected the greater part of the cell-wall undergoes surface growth, but there are many cases known where

growth is confined to one small part of the wall, and this part may be either terminal or, indeed, in any other definite region of the cell. In the first case we speak of the growth as *apical*, and then the growth is distinctly unilateral in relation to the full grown part of the wall ; the other case we term *intercalary* where interpolation of a new region takes place between two zones which are already fully developed. Examples of apical growth are to be found in roothairs, pollen-tubes, fungal hyphae, &c. (HABERLANDT, 1889, REINHARDT, 1892). According to REINHARDT growth is limited to the convex apex of the cell, to the minute projecting region of the cylinder; further, growth is greatest at the extreme apex and decreases gradually backwards. In Fig. 49 two stages in the growth of an apex of a hypha are shown, and corresponding regions are indicated by dotted lines. We see how much the surface *cd* has extended (to *c'd'*), and how little difference there is between the surfaces *ab* and *a'b'*. The best example of intercalary growth is furnished by *Oedogonium*, where the intercalated region is marked off from the older parts in the clearest possible manner. This example will be dealt with in a greater detail later ; meanwhile reference may be made to Fig 51.

Another case of intercalary growth is illustrated at Fig. 50, which shows two stages in the development of stellate parenchyma. The originally closely applied walls of two cells separate from each other at several points, and intercellular spaces, *ii*, appear between them. We may observe thereafter that the cell-wall exhibits further growth practically only opposite the intercellular spaces, while the regions where the two cells are in contact (in Fig. 50, *I*) scarcely grow at all (Fig. 50, *II*).

Surface growth of the cell-wall, like its primitive formation, takes place only in the presence of protoplasm and nucleus, and, as a rule, growth in the wall occurs only where the protoplasm is closely applied to it. This close application of the protoplasm is firmly maintained by osmotic pressure, while at the same time, and due to the same cause, the membrane is kept tense. Owing to the fact that this osmotically-induced tension was observed in the majority of growing cell-walls, and because a certain relation had been noted between pressure and intensity of growth, it was for long considered that osmotic pressure played a mechanical part in surface growth, and the phenomena which occur in artificially formed cells were compared with those in a state of nature. Artificial cells (TRAUBE, 1867) may be readily produced by taking a little gelatine to which sugar has been added and allowing it to exude from the end of a glass pipette submerged in a weak solution of tannin. A precipitation membrane makes its appearance at once on the surface of the drop, a membrane whose characters we have already studied (Lecture II). It is very permeable to water, but quite impermeable both to tannin and gelatine. Under these conditions an osmotic pressure develops inside this membrane which stretches it. One of two things may now happen, either a simple extension of the particles of the membrane by the intercalation of the gelatine solution between those of the membrane, or fine cracks may appear permitting the exposure of the gelatine solution, which, as soon as it comes in contact with the tannin, develops at once a new precipitation membrane of gelatine tannate. Obviously since the formation of new parts takes place quite regularly between the old parts of the wall, the artificial cell will assume the form of a sphere of considerable size.

Is there any likeness between the growth of the precipitation membrane and that of the natural cell ? This question cannot be answered offhand. Of course it is obvious that the membrane in the plant cell is not due to the precipitation of some product of the reaction of two fluids ; still osmotic pressure might, for all that, play a mechanical part in surface growth. This has indeed been accepted as true, and on this basis two theories have been advanced to account for surface growth. According to one view, the cell-wall is simply stretched mechanically by osmotic pressure and that too far beyond the limit

of elasticity (' plastic growth '). In proportion as the surface increases the thickness must decrease, and since in nature no such decrease in thickness can as a rule be observed it must be assumed that simultaneously with this superficial extension a deposition of new layers, or an increase in thickness from within, takes place. This conception, when carefully studied, denies *real* surface growth altogether, and recognizes only a passive stretching and deposition on the membrane. In sharp contrast to this theory is the second, which explains surface growth by assuming the *intercalation* of new materials between those already existent ; osmotic pressure on this view acts mechanically by pulling apart the finest particles of the cell-wall and thus favouring the interpolation of new particles between them.

These two opposing views have long been known as the theories of ' apposition ' and ' intussusception ' respectively, and it is only recently that it has come to be believed that *both methods in all probability occur in nature.* We will illustrate this by reference to a few examples.

In a cell of *Oedogonium* which is about to divide we observe, near one end and closely applied to the inside, a thickening of the wall in the form of a ring-like pad, manifestly composed of two different chemical substances as seen in section (Fig. 51, *I*). The central mucilaginous region of the pad is the first to be laid down (*Schl*), and is covered later by a cellulose layer (*C*) which has the same characters as the remainder of the cell-wall. Apparently, owing to swelling of the mucilaginous core the old cell-wall is torn asunder by a circular crack, and the whole cell is in this way greatly increased in length. The material forming the pad becomes extended so as to form a cylindrical uniting zone between the two older regions of the cell-wall, in such a way that the mucilaginous region forms the external and the cellulose part the internal layer of the intercalated piece. Contemporaneously with the elongation of this interpolated cylinder (Fig. 51, *II*, *III*) there is an obvious decrease in the thickness of the wall, and the whole process gives one the impression that the stretching is purely passive and results from the action of osmotic pressure. On this account *Oedogonium* has been usually regarded as an example of 'plastic growth'. Closer investigation shows us, however, that the case is not quite so simple as it seems. The new membrane at first is stretched in the *longitudinal direction* only, and the *transverse diameter* of the cell remains unaltered or even decreases. Again, it is worthy of note that the growth ceases when the intercalated portion has become quite thin. If the entire growth of this portion depended *only* on plastic extension, one would be led to expect that the stretching must continue, and all the more easily the thinner the membrane became. If *stretching* be the factor in the case at all, then changes in the elasticity of the membrane must occur during that process, calculated to render the membrane gradually *less extensible.* Even of this simple and apparently purely mechanical growth phenomenon we are far from having arrived at a satisfactory explanation. It would seem to us as if the physiological aspect of the case has not as yet received the attention it deserves.

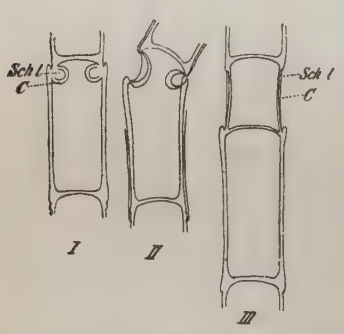


Fig. 51. Cell division in *Oedogonium borisianum* (after HIRN). *Schl*, the gelatinous pad; *C*, the cellulose covering of the pad.

BERTHOLD has observed intercalary growth in other Algae also, which in many respects suggests that seen in *Oedogonium*, e. g. in a member of the Confervaceae. The cell structure of this alga is illustrated schematically at Fig. 52. Each cell consists of two pieces, ⊼-shaped in longitudinal section, each becom-

ing thinner towards the middle of the cell and having these thinner regions overlapping. The protoplasm next deposits a median band of membrane, thick in the centre and thinning off towards either end. Union of the two constituents renders the wall of the cell of equal thickness throughout. When the cell starts growing the overlapping borders of the external membrane separate and the inner membrane comes up more and more to the surface of the cell-filament. After a transverse wall has been formed this inner wall takes on the H-form and new inner membranes appear in each daughter-cell. Similar observations on other Algae have been made by many other investigators (compare BERTHOLD, 1886, and KNUT BOHLIN, 1897). The difference between this case and *Oedogonium* lies chiefly in the fact that the deposition layer does not grow so suddenly in the present case, and hence the idea of purely mechanical stretching is excluded.

Phenomena similar in principle have been demonstrated as taking place in apical as well as in intercalary growth. Thus SCHMITZ (1880) and STRASBURGER (1882) have observed at the growing apex of *Bornetia secundiflora*, one of the Florideae, a special kind of lamination which is figured at Fig. 53. The growing point is made up of successive lamellae of limited thickness which increase in superficial extent and burst through similar older lamellae, which thus become bevelled off at a certain distance from the apex. NOLL (1887) successfully stained the cell-walls of *Derbesia*, *Caulerpa* and other marine Algae with prussian-blue and thus differentiated these layers from those succeeding them. These experiments make it certain that deposition of new lamellae takes place at the growing point, by whose superficial growth the older lamellae are burst. We may certainly assume from a study of such observations that the separate lamellae then exhibit no further growth where they have been torn through, or do not, at least, grow as vigorously as the newly-formed lamellae. The older lamellae are, doubtless, *passively* stretched, but whether the young lamellae grow passively also cannot be decided from the experiment. If in this and other similar cases the young cell-wall also suffers passive stretching we should naturally attribute it to osmotic activity; but it is of importance to note that this osmotic pressure is insufficient of itself to stretch the membrane plastically, since it is known that much greater pressures are unable to extend the cell-wall beyond its limits of elasticity (PFEFFER, 1892, 241). Again, we never find membranes in the living cells which are stretched beyond the limits of their elasticity. We may assume, however, with NOLL (1895) that plastic stretching is possible without overstepping the bounds of elasticity. It should be remembered that a bent wooden bow gradually unbends, a fact which can be due only to an internal concentric layering. In this unbent condition, however, the bow at any moment can be again elastically bent. Similarly, in the cell-wall stretched by turgor pressure, an unbending and even a plastic extension might arise without the wall becoming thereby inelastic. It is often assumed that the *protoplasm* has an effect on the elasticity of the membrane, but we have no grounds for such an assumption.

Fig. 52. Structure of the cell-wall of *Microspora amoena*. After KNUT BOHLIN (1897, Pl. I, Fig. 18). × 300.

Fig. 53. Lamination in the apical cell of *Bornetia secundiflora*, × 75. After STRASBURGER (1882, Pl. IV, Fig. 55).

It was for long believed that surface growth in the cell-wall arose only by deposition of lamellae and plastic stretching of these by turgor pressure, but more recently many arguments have been advanced against this view; moreover, there are individual cases which have been thoroughly examined, and to which this explanation cannot be rightly applied. There is first of all the apical growth

of fungal hyphae and roothairs, studied by HABERLANDT (1889), ZACHARIAS (1891), and REINHARDT (1899). So long as these structures are allowed to grow without any interference, no deposition of new lamellae and bursting of older layers can be observed in them. Such a negative result taken by itself certainly does not help us much, but we may obtain an explanation of the mode of growth, such as has been demonstrated in *Bornetia* and *Derbesia*, if the *cessation of growth* be studied. Thus ZACHARIAS has shown that the roothairs of *Chara*, when placed in water in which *Chara* has never been grown, at once cease to grow. When, after a certain interval, growth begins again, one sees that the parts of the cell-wall which previously had shown power of growth, have now lost that power and are burst asunder by newly-deposited layers. REINHARDT observed the same phenomenon in roothairs which had been plasmolysed. Although REINHARDT perhaps goes too far in assuming that in *Bornetia* the bursting of a layer always follows a previous interruption in growth, one is forced to admit that in the cases studied by REINHARDT and ZACHARIAS, growth may be normally carried on without successive deposition and bursting of lamellae. The burstings must take place in these examples with quite remarkable frequency; according to REINHARDT at least once or twice a minute in the case of fungus cells. It is scarcely possible that the mode of growth can in this case be other than by *intussusception*, i.e. by the interpolation of new wall substance between the older molecules. An observation of ZACHARIAS, following directly on NOLL'S experiments, supports this view. He succeeded in introducing congo-red, under certain conditions, into the membrane of the roothair without occasioning an interruption in growth, and was able to determine clearly that the coloured apex gradually lost its colour during surface growth, while older portions of the same wall retained it. It was very apparent that in this case there was no bursting of the coloured lamellae as in NOLL'S experiments, but rather an interpolation of materials between the minute older particles. This result admits of more than one interpretation, and hence we cannot conclude *with certainty* that the mode of growth in *Caulerpa* in NOLL'S experiments is conditioned only by the colouration with prussian-blue and by artificial interruption of growth.

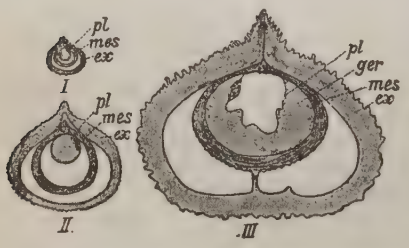


Fig. 54. Development of the megaspore of *Selaginella helvetica*. After FITTING (1900, Pl. II, Figs. 10, 11, 12). *I–III*, successive developmental stages, similarly magnified (× 180). *ex*, exosporium; *mes*, mesosporium; *pl*, protoplasm; *ger*, coagulum between the protoplasm and the mesosporium.

Further important evidence in favour of surface growth by intussusception has been brought forward by ASKENASY (1890) and STRASBURGER (1889). It is impossible to quote all these proofs at present, and we will, therefore, note only a special case which has been recently drawn attention to by FITTING (1900). The young spore of *Selaginella* possesses two chemically different membranes, the exosporium and the mesosporium (Fig. 54, *ex* and *mes*); they are separated from each other by a fluid containing very little solid material. As the spore increases in size (Fig. 54, *I–III*) these lamellae retain their relative positions but increase correspondingly in *surface* growth and in *thickness*. In an ordinary cell-wall the *innermost* lamella at all events can increase in surface as a result of plastic stretching and simultaneous deposition of new layers from the protoplasm; the outer layers, however, must become *thinner* as a result of plastic extension. In the spores of *Selaginella*, however, the *inner* lamellae are quite debarred from any possible deposition of new layers, since the protoplasm is *contracted* into a sphere within the mesosporium (Fig. 54, *II*), and touches the membrane at most at *one* spot only. Between the protoplasm and the

mesosporium there is a fluid from which a coagulable substance may be precipitated by alcohol (*III, ger*). The growing membrane is in contact with fluid on *both sides*, from which it obtains the necessary nutrients and thus grows in extent *without being stretched by osmotic pressure.*

We are doubtless dealing here with an extreme case, since in general the membrane grows superficially *only so long* as it remains in contact with protoplasm ; when the protoplasm is withdrawn from the wall by plasmolysis it tends to excrete a new membrane on its outer surface. Why the sporogenesis in *Selaginella* and *Isoetes* shows deviations from the normal has not as yet been explained, but that this difference exists cannot be doubted after FITTING'S researches. The behaviour of the spore-walls in relation to osmotic pressure is not so extreme, since it has been observed in other cases that a vigorous surface growth can take place without an increase in turgidity, and that growth in general may go on although turgor be reduced. Thus PFEFFER has shown (1893) that the growth of the walls of the root-cells continues after osmotic pressure has been more and more reduced by appropriate means, the cell-wall finally becoming entirely, or almost entirely, contracted. In PFEFFER'S experiments this retraction was effected by a sheath of plaster of Paris, but KOLKWITZ (1896) showed that similar conditions may also occur in nature ; the cells of the medulla of *Helianthus*, for example, exhibited growth at their ends after they had been squeezed by the expansion of the vascular bundles.

Such observations render it probable that the part played by turgor in these and other cases is not merely mechanical. To effect *plastic stretching* an expansive force is certainly necessary, and this force must be that of turgor ; turgor is, however, quite unnecessary for growth by intussusception. This pressure is indeed quite insignificant in comparison with molecular forces, such as that of crystallization for example. Thus crystals of calcium oxalate may originate and grow *in* the walls of certain cells (PFEFFER, 1892, 250 ; MÜLLER, 1890), and these must have overcome the cohesion of the particles of the cell-wall. Intussusception may therefore be best compared with phenomena of this kind.

One would think there ought to be no difficulty in settling experimentally the part played by turgor in this process. If we place a turgid cell in an osmotically active solution the expansion of the wall is thereby reduced ; the *stoppage* of growth which follows is, however, by no means a purely physical result of the reduction of tension, but is due to a complicated stimulus action. Further, we may increase the tension of the wall by reducing the osmotic value of the external medium (e. g. in marine Algae), but in this case also the alteration acts as a stimulus and growth ceases in consequence. In both cases, growth after a time recommences, but in the interval a readjustment of osmotic pressure takes place ; in short, it is a matter of great difficulty to determine experimentally the relations between growth and turgidity. Theoretically we may say that turgor, though it does not act directly by supplying the *energy* necessary for growth, is nevertheless necessary for it. It may be considered ' as a *condition* of growth, just as in an analogous manner a certain degree of heat may be so regarded ' (PFEFFER, 1892, 219). Growth, however, does not appear to be proportional to turgor pressure ; in many cases turgor appears to be regulated by growth rather than growth by turgor (COPELAND, 1896).

Concomitantly with or after surface growth of the membrane *growth in thickness* takes place. Very frequently this may be readily shown to be due to the deposition of new lamellae, in other words, to *apposition.* If it continues after surface growth ceases this apposition results in a lessening, and, finally, in partial occlusion of the lumen of the cell; hence this type of growth takes place *in opposition to* the pressure of the cell contents. We are acquainted, however, with other well-established observations which prove that a marked increase in

volume takes place in lamellae laid down by apposition, and also in cases where these lamellae are separated from protoplasm by other layers. Growth in thickness can thus be produced by intussusception also. We will content ourselves with one example, viz. the cell-wall of *Gloecapsa alpina*, which has been carefully investigated by NÄGELI (1858) and CORRENS (1889). When the cells of this alga divide each daughter-cell forms a new cell-wall of its own. The original wall, however, now separated from the protoplasm by these new walls, increases also both in thickness and in extent as shown in Fig. 55, *II*. CORRENS's measurements and arguments show that this growth is explicable only on the assumption of an interpolation of organic material.

Not infrequently the greatly thickened membrane remains completely homogeneous, but most usually we can make out a concentric lamination in it which may be due to different causes (CORRENS, 1891). The successive layers may be differentiated *chemically*, or only by the *amount of water* they contain. In the latter case the lamination is due to the same cause as in starch grains; but the causal connexion is no more clearly explained in the one case than in the other. We need not, however, enter in further detail here as to the structure and growth of starch grains, although these bodies have taken a foremost place in all discussions on growth, and will always have a historic interest as being the basis of NÄGELI's (1858) theory of intussusception. Thanks to the investigations of MEYER (1881 and 1895) and of SCHIMPER (1881) we now know that the growth of a starch grain is effected by the external *apposition* of new material from without, and that the increase in size of the grain takes place in the same way as increase in size of a crystal or a spherocrystal. In a certain sense starch grains are rather subjects for crystallographical than physiological study. We say 'in a *certain* sense' intentionally, since although growth by *apposition* has been definitely *proved* to occur in them the occurrence of *intussusception* also is not completely *excluded*.

Fig. 55. *Gloeocapsa alpina*. After CORRENS (1891, Pl. 15). *I*, two-celled stage; *II*, eight-celled stage. The most external wall (left clear) is common to all the cells and has grown greatly in thickness.

One point of similarity between the starch grain and the cell-wall must be noted. Many cases in which we were in the past accustomed to assume growth in the cell-wall by intussusception have now been shown to be due to apposition; similarly cases of increase occur due to *supplementary* intussusception, and hence it was customary in any generalization to give 'apposition' or 'intussusception' as alternatives. At the time when there was a reaction against the NÄGELI-HOFMEISTER theory of intussusception one had become accustomed to regard the cell-wall as a non-living structure, and to compare it with the shell of a mollusc. HOFMEISTER (1867), on the other hand, held that the membrane was alive and ascribed to it all the characters we now associate with protoplasm only. When cases became known which militated against the universality of apposition, and which rather suggested the activity of a living structure, attempts were made to explain the situation by assuming an infiltration of the protoplasm into the cell-wall, and so to account for its apparent vitality (WIESNER, 1886; STRASBURGER, 1889). The existence of this hypothetical plasma in the wall has never as yet been demonstrated (CORRENS, 1894), and so perhaps we may come back once more to HOFMEISTER's conception and regard the membrane itself as *living*, although certainly not independent life as we understand it in protoplasm, since there is no doubt whatever that protoplasm may grow without a cell-wall, but not a cell-wall without protoplasm. But the membrane is perhaps alive in so far as it can exhibit the power of 'assimilation in the real sense of the word' (p. 259), being able itself to form new wall material out of definite but as yet unknown substances provided by the protoplasm. If this be

so we should have a living substance which contained no nitrogen. It is unnecessary for us to trace any further the consequences of the acceptance of this hypothesis; it will be preferable to bring forward an example where we may most readily consider such an active formative power in the cell-wall, viz. the bands, spines, and other sculpturings formed on the surface of spores and pollen-grains (WILLE, 1886; STRASBURGER, 1889). Here we have to deal not only with a simple thickening of a previously existent cell-wall, but with *differential thickening at special places*, resulting in a *configuration* fixed by heredity. We meet with such a case in the exosporium of *Selaginella galeotti* (FITTING, 1900), after the exosporium has been separated first from the mesosporium and later on from the protoplasm. The extent to which the protoplasm has retracted renders the hypothesis of migration of protoplasm into the wall, in this case, especially unlikely.

We have dwelt too long already on the question of the growth of the cell-wall, although we have left unmentioned many questions which call for investigation, but we may refer, before leaving the subject, to one further point only, the *cessation* of growth. If we limit ourselves to surface growth, we may distinguish cells which theoretically grow on for ever, unless destroyed, from those which continue to grow only for a definite time (p. 273). The question as to what brings about this cessation of growth has been very variously answered. Looking only at the great thickness and varying chemical characters of the fully-developed cell-wall attempts have been made to give a purely mechanical explanation of how such a wall has no further power of growth. As a matter of fact, however, in the first beginnings of lateral branches, and also after wounds, completely adult walls may start growth afresh, and we often see very thick-walled cells still capable of growth (KRABBE, 1887), while thin-walled cells do not show that power. The directing influence inducing or stopping growth must arise from the *protoplasm*, for all growth phenomena are regulated by the living organism. Such regulating processes meet us at every turn, wherever growth and formation in plants are closely examined. It is impossible to say with certainty whether definite organs in the cell, the nucleus more especially, play any special part in this phenomenon. We know for certain (TOWNSEND, 1897) that the protoplasmic complex can manufacture a membrane only when it is provided with a nucleus, or when it is connected by protoplasmic strands—however delicate—with protoplasm which contains one. It does not follow, however, that cell-wall formation is a special function of the nucleus, for the nucleus is no more able to form a cell-wall without protoplasm than protoplasm without a nucleus. HABERLANDT (1887) has pointed out that the nucleus frequently approaches the region of the wall which is actively growing, and he concludes from that that the nucleus has some special duties to perform in relation to the formation of cellulose. At the same time cases are known in which the nucleus obviously occupies *other* situations, and these militate against the correctness of this conception.

Reviewing the whole position we may say that the growth of the cell-wall takes place in a *variety of ways*. All theories which involve the acceptance of only one mode of growth must be rejected; for even in those cases where growth appears purely mechanical we cannot do without the complicated and invisible influence of protoplasm. Protoplasm administers the stimulus which induces both construction and growth of the cell-wall and it determines finally when they shall cease. Protoplasm determines the degree of osmotic pressure, which must decrease with every increase in the size of the cell unless the osmotically active material be renewed, and thus also influences the capacity for extension of the cell-wall. Further, although we may speak of the cell-wall in individual cases, or in general, as possessing life, still that does not alter its dependence on protoplasm.

Growth of the cell is associated frequently but not always with cell division. There are plants, such as the Siphoneae and Mucorinae, which attain to quite

considerable dimensions and complicated form, and which still possess the characters of a single cell ; but the great majority of cells divide after reaching a certain size. In a typical case this takes place in the following manner. The first alteration to be observed is in the *nucleus*. In consequence of complex internal processes, shown in Fig. 56, part of the *chromatin* first of all forms a much twisted thread (*2*), which afterwards breaks up into a definite number of segments, the so-called chromosomes (*3, 4*). In plants there are usually several such chromosomes, which after taking on a U-shape, aggregate at the equator of the nucleus. Each chromosome then splits longitudinally (*5, 6*), and the halves so formed separate from each other in opposite directions (*8, 9*). Each group then exhibits a fusing of the chromosomes into a network similar to that present in the original nucleus (*10–12*). Thus there arise two daughter-nuclei separated

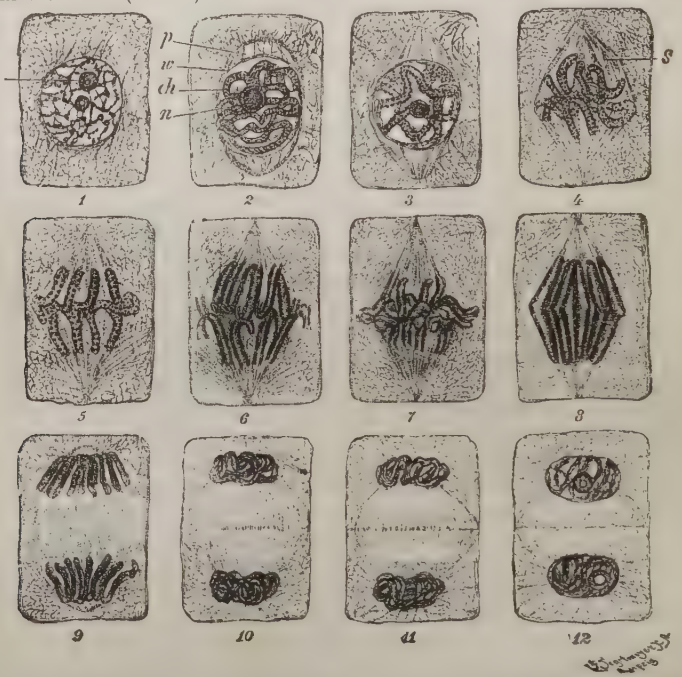


Fig. 56. Mitosis and cell division in embryonic tissue. Somewhat diagrammatic. *n*, nucleolus; *p*, polar plates; *w*, nuclear wall; *ch*, chromosomes; *s*, spindle fibres (× 600). From the Bonn Textbook.

from each other by an interval. The stages *1–6* in the figure are collectively spoken of as the *prophase*, *7–9* as the *metaphase*, *10–12* as the *anaphase*. In the stage represented at Fig. 56, *4* we observe a system of fine threads stretching from one ' pole ' of the nucleus to the other. They arise either from the ground substance of the nucleus (ZACHARIAS, 1888) or, according to many authors, they enter into the nucleus from the surrounding cytoplasm (STRASBURGER, 1888). Certain of these spindle threads (' contractile threads '), by their contraction, pull the chromosomes towards the poles (Fig. 57), the remainder persist as connecting links between the two daughter-nuclei and form a basis for the formation of the cell-wall. In the equatorial plane of the division figure (Fig. 56, *10, 11*) there arise thickenings on the spindle threads, so that the individual filaments approximate laterally at this point and form a protoplasmic wall (*12*), the so-called cell or equatorial plate, which cuts the spindle medianly in two. The cell-plate splits afterwards into two lamellae between which the cell-wall is excreted (STRASBURGER, 1898).

If now, as is often observable, the nuclear spindle broadens, so that it occupies the whole diameter of the cell, the new cell-wall placed at right angles to the old membrane divides the cell at once into two halves. A 'simultaneous' cell-wall formation of this sort occurs especially in narrow cells. It is not confined to such however, but occurs also in broad cells, when the nuclear spindle extends across the whole cavity of the cell (Fig. 56, *12*). In addition to the simultaneous type we also not infrequently meet with a gradual or 'succedaneous' formation of the cell-wall. The new wall, as in *Spirogyra*, may extend inwards from the mother cell-wall in the form of a plate or ridge, with a central aperture which gradually becomes contracted (Fig. 58), or the formation of the wall may commence at a definite spot on the wall of the mother cell and slowly grow across it (Fig. 59). In this latter case the nuclear spindle moves forward as the new cell-wall gradually crosses the mother-cell. After the wall has been formed, the remainder of the nuclear spindle left over in the two daughter-cells disappears. In a few cases, such as that of *Oedogonium*, the complete wall is formed within the cell and unites later with the older cell-wall (compare HIRN, 1900); as a rule, however, the wall is built out from the spot where it originates.

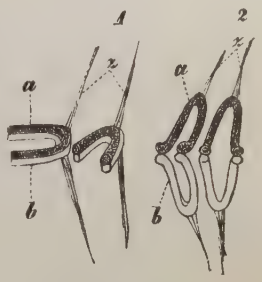


Fig. 57. Diagram to illustrate the separation of the chromosomes. *z*, spindle threads; *a* and *b*, the two longitudinal halves of the chromosome. (From the Bonn Textbook.)

The division of the mother-cell takes place in a quite definite manner, and the laws governing cell division were first, at least in part, elucidated by HOFMEISTER (1867) and SACHS (1878-9), but the sequences were worked out more accurately by ERRERA (1886) and BERTHOLD (1886). Both authors found that

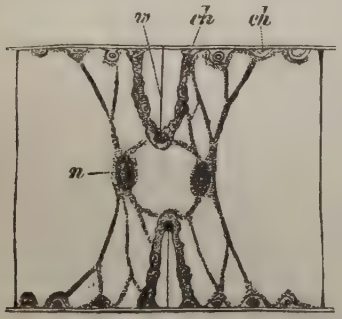


Fig. 58. Division of a cell of *Spirogyra*. *n*, daughter-nucleus; *w*, partition wall; *ch*, chloroplast (× 230). From the Bonn Textbook.

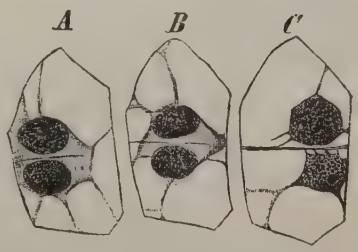


Fig. 59. Cells of *Epipactis palustris* in three successive stages of division. After TREUB, from the Bonn Textbook (× 365).

in the majority of cases the new walls were placed across the old ones exactly as would be the case with a soap film. The laws of arrangement of liquid lamellae have been closely studied and we know that these lamellae always show what are termed 'minimum surfaces'. If a soap film be stretched diagonally across a cube (Fig. 60, *1*) it will shift its position until it has attained a minimum area, that is to say, in this case, until it has divided the cube into two parallelopids (*2*). If we extend a partition in the cube quite close to one of the walls and parallel to it, it shifts itself and bends until it has cut off a segment of the cube (Fig. 60, *3*). Similarly in the plant-cell partition walls appear, sometimes straight, sometimes curved, but to study the details of these would take us too far. As we have already said, they exhibit in the majority of cases minimum surfaces, save

that exceptions are known, as, for example, in cells which divide longitudinally when one would expect transverse division (e. g. cambium cells).

The occurrence of cell-walls as surfaces of ' minimum area ' might be explained most simply by assuming that the freshly formed wall is in a liquid condition. ERRERA (1886) is responsible for this view, though observation would lead us to an opposite conclusion. More recently, WILDEMAN (1893) has suggested that it is sufficient if the cell-wall be in a liquid condition for one moment only and then becomes rigid. Such a hypothesis cannot be denied, at least in the case of simultaneous cell-wall formation, but it does not lend itself to the explanation of the *succedaneous* type. There, undoubtedly, one part of the wall already existed

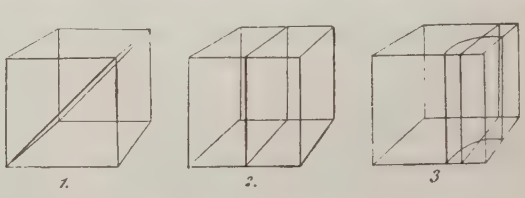


Fig. 60. After BERTHOLD (Protoplasma mechanik).

in the solid state, while the rest is not even deposited. What is it, then, that makes the cell-wall grow on in a definite direction? WILDEMAN has shown that even a liquid lamella may increase by *succedaneous* accretions. If one places a soap film within a rectangle made of iron wire (Fig. 61, *1*) into which runs a silk thread attached at *a* and *b*, the ends of which pass through a straw suspended in the soap film, and if the lamella be pierced between *a* and *b* and the threads, one obtains the condition represented at Fig. 61, *2*. If now the ends of the thread be pulled (as indicated by the arrows in the figure) the soap film gradually grows from right to left till it fills the whole rectangle. There are great difficulties, however, in applying this experiment to dividing cells. What corresponds in the cell to the silk threads and to the frame? No answer can be given to this question; but without the threads and the framework no succedaneous growth of the lamella is possible.

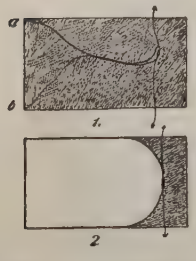


Fig. 61. DE WILDEMAN'S experiment.

Although at first sight one is tempted to refer cell division to the laws governing the equilibrium of liquid membranes, still it is not possible for us to accept this explanation. We must content ourselves with pointing out the great similarity between these two sets of phenomena and confessing that the reasons for this comparison are still far from apparent.

After the sketch we have now given of cell division it will be obvious that we must attribute very important functions in that process to the nucleus, and especially to the phenomena of mitosis. We must not, however, overrate their importance, for we must remember that quite normal cell division may take place without mitosis, and indeed without segmentation of the nucleus at all. NATHANSOHN'S (1900) and WASILIEWSKI'S (1902) researches show that by employing certain anaesthetics (ether, chloral hydrate) the normal nuclear division (mitosis) taking place in the cells of *Spirogyra* and of the root apex of *Vicia faba* may be transformed into the so-called direct nuclear division, where the nucleus divides into two parts without any formation of chromosomes or nuclear spindle and between these two nuclei a partition wall is formed. [WISSELINGH (1903, Bot. Ztg., 61, 201) has raised objections to this interpretation of NATHANSOHN'S discoveries. The controversy between these authors (Bot. Ztg., 1904, 62, II, 17) suggests the necessity for a re-investigation of the whole question. Doubt

has also been thrown on WASILIEWSKI's work by the experiments of NĚMEC (1904, Jahrb. f. wiss. Bot. 39, 645). At present it cannot be affirmed with certainty whether 'amitosis' does take place under the conditions mentioned above; still less certain are we of the results contingent on it.] The daughter-cells, which are produced in this way, behave normally and divide mitotically later on.

Cell division also occurs without any visible bi-partition of the nucleus. This is the case, for instance, in the multi-nucleate cells of *Cladophora*; division takes place as in the case of *Spirogyra*, but the partition wall is not formed on a nuclear spindle. The cells of Cyanophyceae and Bacteria also exhibit normal division, yet they appear generally to contain no nuclei. Further, GERASSIMOFF (1899) succeeded in so altering the division process in *Spirogyra* that *one* of the daughter-cells contained two nuclei, the other none; and although in this case also the transverse wall could not be formed on the spindle still it showed no difference in appearance to the normal wall. GERASSIMOFF (1901) also succeeded in some experiments in so influencing the cells during division that the division of the *nucleus* was suppressed while *cell* division still went on; one daughter-cell thus had *one* nucleus while the other had *none*. The cell containing the nucleus and its descendants were distinguished for a long time by their remarkable size, and attained a great length before proceeding to divide afresh. The nucleus has also a certain influence on the *specific amount of division*, which depends also on many internal and external factors. We cannot go into these here and will content ourselves with noting that *one* internal factor, viz. the *function* of the cell, influences in a remarkable way the amount of division it undergoes. The varied size of different cells in the complex plant depends on this fact; but the same phenomenon may be observed in quite simple organisms also. Compare, for example, the difference in size between the ordinary vegetative cells and the sexual (male) cells in *Oedogonium*. In spite of its variability the amount of division in cells is in general a character of the species. Thus cells as small as those which occur in Bacteria are unknown in Phanerogams; cells as large as those of *Caulerpa*, are found in flowering plants only in isolated positions and adapted to carrying out special functions (laticiferous tubes).

When a cell has reached a certain size it divides, and so attains once more its normal size, and *division into two* as a rule leads to this result. Often, however, a formation of many cells takes place, four or even more cells being formed from *one*. To give illustrations of this phenomenon and to describe the arrangement of the walls in such cases would lead us too far, and so we may merely refer to detailed descriptions of cell morphology.

Although in general a certain amount of growth precedes cell division still this is not a universal *condition* of that division. Certain embryonic cells divide into numerous small cells without exhibiting any actual growth; thus the ovum of *Fucus*, the spores of many Hepaticae and Fungi, the segments of the apical cell of *Stypocaulon* are illustrations in point, but of these we shall speak in our next lecture.

Bibliography to Lecture XXI.

ASKENASY. 1890. Ber. d. bot. Gesell. 8, 61.
BERTHOLD. 1886. Studien über Protoplasmamechanik. Leipzig.
BOHLIN, K. 1897. Bihang svenska Vet. Akad. Handl. 23.
COPELAND. 1896. Einfl. d. Temperatur und des Lichtes auf den Turgor. Diss. Halle.
CORRENS. 1889. Flora, 72, 298.
CORRENS. 1891. Jahrb. f. wiss. Bot. 23, 254.
CORRENS. 1894. Ibid., 26, 587.
CORRENS. 1898. Bot. Ztg. 56, II. Abt. Sp. 221.

Driesch. 1901. Die organischen Regulationen. Leipzig.
Errera. 1886. Bot. Centrbl. 34, 395.
Fitting. 1900. Bot. Ztg. 58, 107.
Gerassimoff. 1899. Bullet. Soc. d. Naturalist. de Moscou, 1899, 220.
Gerassimoff. 1901. Ibid. 1901, 185.
Haberlandt. 1887. Funktion u. Lage des Zellkernes. Jena.
Haberlandt. 1889. Oestr. Bot. Ztg. (Nr. 3).
Hirn. 1900. Monographie u. Ikonographie der Oedogoniaceen. Acta Soc. scient. Fenn. 27.
Hofmeister. 1867. Die Lehre v. d. Pflanzenzelle. Leipzig.
Kolkwitz. 1896. Fünfstück's Beitr. z. wiss. Bot. 1, 246.
Krabbe. 1887. Jahrb. f. wiss. Bot. 18, 346.
Meyer, Arthur. 1881. Bot. Ztg. 39, 841.
Meyer, Arthur. 1895. Untersuchungen über die Stärkekörner. Jena.
Müller. 1890. Entstehung von Kalkoxalatkristallen in Zellmembranen. Diss. Leipzig.
Nägeli. 1858. Pflanzenphys. Unters. 2. Die Stärkekörner, p. 281.
Nathansohn. 1900. Jahrb. f. wiss. Bot. 35, 48.
Noll. 1887. Abh. d. Senkenbergschen Gesell. 15, 101.
Noll. 1895. Flora 81, 65.
Pfeffer. 1892. Abh. math.-phys. Cl. K. Sächs. Gesell. d. Wiss. 18.
Pfeffer. 1893. Druck u. Arbeitsleistung (Abh. math.-phys. Cl. K. Sächs. Gesell. d. Wiss. 20).
Reinhardt. 1892. Jahrb. f. wiss. Bot. 23, 479.
Reinhardt. 1899. Festschr. für Schwendener. Berlin.
Sachs. 1878–9. Arb. bot. Instit. Würzburg, 2, 46; 2, 185.
Schimper. 1881. Bot. Ztg. 39, 185.
Schmitz. 1880. Verhandl. naturw. Verein d. Rheinlande, 36.
Strasburger. 1888. Kern- u. Zellteilung im Pflanzenreich. Jena.
Strasburger. 1882. Ueber den Bau u. d. Wachstum der Zellhäute, p. 189. Jena.
Strasburger. 1889. Histolog. Beitr. Heft 2. Jena.
Strasburger. 1898. Jahrb. f. wiss. Bot. 31, 511.
Townsend. 1897. Jahrb. f. wiss. Bot. 30, 484.
Traube. 1867. Archiv f. Anat. u. Phys. p. 87.
Wasiliewski. 1902. Jahrb. f. wiss. Bot. 38, 377.
Wiesner. 1886. Sitzungsber. Wiener Akad. math.-nat. Klasse, 93.
Wiesner. 1892. Die Elementarstruktur u. das Wachstum d. leb. Substanz. Wien.
Wildeman. 1893. Mém. couron. par l'acad. Belg., 4to, 53.
Wille. 1886. Ueber d. Entw. der Pollenkörner d. Angiospermen. Christiania.
Zacharias. 1888. Bot. Ztg. 46, 33.
Zacharias. 1891. Flora, 74, 466.
Zimmermann. 1893. Beitr. z. Morph. d. Pflanzenzelle, 1, 209.

LECTURE XXII

THE GROWING POINT

The daughter-cells which arise by cell division may either separate from each other or they may remain connected. If they separate, the plant in question is unicellular in the truest sense of the word, in the other case cell aggregates arise in the form of cell filaments, cell plates and cell bodies according as growth takes place in one, two or three dimensions of space. If all the cells of such an aggregate are like each other, and each cell is physiologically quite independent, the distinction between those which take the form of such aggregates (colonies) and those which are strictly unicellular is not well marked, and numerous transitions between these conditions are to be found; indeed one and the same plant may, according to external conditions, exhibit a unicellular form or a colonial form.

The two sister-cells are not, however, always morphologically and physio-

logically alike; on the contrary, we meet with greater and greater differences between the individual cells as we ascend in the organic scale, and these differences are indicative of their physiological activities. Every cell has no longer the same function; *division of labour* has appeared, and, as a consequence, the individual cells have lost their physiological individuality and have become mutually dependent on each other; in a word, they are no longer capable of individual and isolated existence and can carry out their functions only when united into a 'differentiated' complex. The first of these differentiations is the separation of a series of cells capable of continued growth and division from cells which, having reached the adult state, are in a resting condition, and since these merismatic cells are not distributed irregularly among the resting cells, but occur in the simplest case at *one* end of the plant body, it comes about that this primary differentiation results in the formation in the plant of two poles, a *base* and an *apex*. Let us study, as a simple example, a cell filament, composed of a row of cylindrical cells, *a*, *b*, *c*, &c., all fully developed. The apex is occupied by the only cell capable of further growth (the apical cell *s*), differing in form from the rest. When the apical cell has reached a certain length it divides, and the daughter-cell *d*, lying next to *c*, becomes a resting cell while the other, *s'*, remains as an apical cell, and so the process goes on indefinitely. We may term the apical cells *embryonic* cells, and the cells derived from them *somatic* cells. Hence we may describe the higher plants which exhibit this differentiation as somatophytes, whilst the lower forms, which possess no fully developed region, i.e. no 'soma', may be termed asomatophytes (PFEFFER, Phys. II, § 2). Although the transition from embryonic to somatic, or fully developed, cells takes place gradually, still the contrast between them is sufficiently distinct. It will be better to speak in this case not of cells but of 'substance', embryonic substance and somatic snbstance, for the embryonic substance need not be confined to *one* cell, as in the example we have taken, but may exist in *many* cells or may be limited to a *part of one* cell. The regions where the embryonic substance is found in a plant may be styled, following the older nomenclature, the '*growing points*'. The growing point, however, does not always lie at the free *apex* of a plant body; it may occur at the *base* or *between* two somatic regions. We have, therefore, to distinguish *terminal*, *basal*, and *intercalary* growing points.

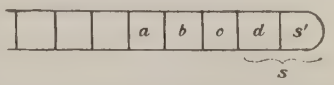


Fig. 62. Diagram of a cell filament with an apical cell, *s*.

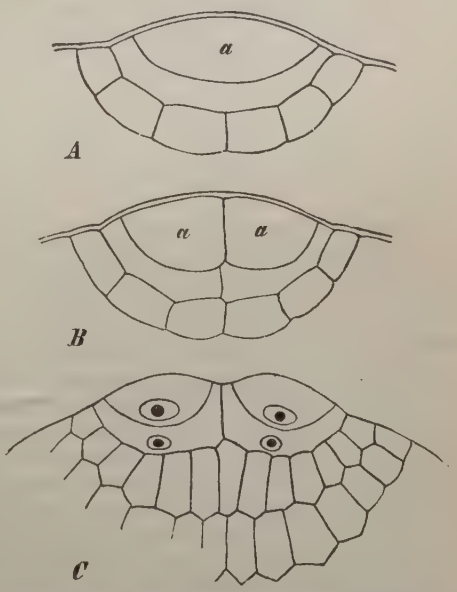


Fig. 63. Dichotomy in the growing point of *Dictyota*. *a*, apical cell (× 500). After DE WILDEMAN in the Bonn Textbook.

In the simplest case the entire activity of the growing point is devoted to the *elongation* of a previously existing body, but when the plant is *branched* then the growing point has also to provide for the production of these outgrowths.

The formation of branches at the growing point may take place in one or other of two ways. In many plants two branches are formed at the same time; the previous direction of growth is abandoned, and instead of the prolongation of the plant body in a straight line a *forking* or *dichotomy* appears. The alga *Dictyota* may be cited as an example of this type. Here the dichotomy is effected by the longitudinal segmentation of the apical cell (*a* in Fig. 63). Similar cases of dichotomy are found in many liverworts; in higher plants, on the other hand, this method is seldom met with (shoots and roots of *Lycopodium* and *Selaginella*). In the highest plants generally, and also very frequently in the lower plants, another type, viz. *lateral branching* occurs, that is to say, in addition to the elongation of the body in a straight line, there are formed also lateral projections on the growing point, so that we may distinguish an *axis* and its *lateral branches*. As a rule, lateral branches are formed not *once* only or *one at a time* on the growing point, but they occur for the most part in *great numbers* continuously or at periodic intervals, and their developmental succession is quite definite, in fact, *progressive*. The youngest are nearest to the growing point, and the further we get from it the older do we find the lateral branches to be. According as the growing point is situated at the fixed

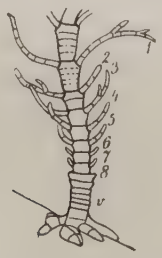


Fig. 64. *Arthrocladia villosa*, showing a basal growing point *v*; 1, 2, 3, &c., indicate the basipetal development of the lateral branches. After FALKENBERG (1882, Art. Algae in SCHENK'S Handbuch der Botanik).

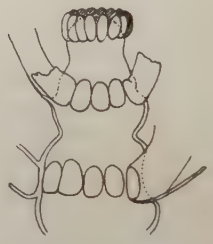


Fig. 65. *Dasycladus clavaeformis*. Growing point with three whorls of branches. After CRAMER (1887) (× 40).

base or the free apex of the axis, the progressive development is basipetal or acropetal. Fig. 64 shows an example of *basipetal* growth, while the more ordinary *acropetal* development is illustrated at Figs. 65 and 66.

Definite relations of symmetry, purely geometrical in character, exist in the *arrangement* of the lateral organs at the growing point, which we must study more carefully. We are able to distinguish radial, bilateral, and dorsiventral growing points. In radial symmetry we find *three* or more longitudinal regions exactly like each other, in bilateral symmetry there are only *two* such, in dorsiventral symmetry only *one* plane.

To take an example, Fig. 65 shows us a radially symmetrical growing point of *Dasycladus*, a member of the Siphonaceae. This seaweed consists of one long cell fixed at the base and elongating at its apex. The lateral appendages are arranged in regular whorls on cask-like swellings of the axis. Immediately below the apex of the growing point arises a whorl of fourteen branches, and at a certain distance behind are two successively older whorls each composed of the same number of branches, whose bases only are shown in the figure. The successive whorls alternate (NOLL, 1896), that is to say, each of the branches of the whorl above stands over the gap between each pair of branches of the whorl next below. We find this alternation of branches to be the general rule in whorled arrangements of lateral organs, whether we are dealing with simple projections from a single cell, as in *Dasycladus*, or with complex organs such as

the leaves of higher plants. Interruptions in the alternation are (as in *Dasycladus*), however, always found if the number of the branches in the whorl be altered and an increase takes place in proportion to the vigour of the plant. The whorls in *Dasycladus* are especially worthy of notice in this relation because they are laid down at a considerable distance from each other. The case figured at Fig. 66 is much commoner; here also the whorls alternate, but they are crowded so closely together that the lateral branches in successive whorls are in actual contact with each other.

Another type of arrangement is met with when we pass from the 'whorled' to the 'spiral' distribution of lateral members. The characteristic feature of this type is that at any definite level on the axis only *one* lateral branch is developed, not two or more. We will select a very simple case and represent it diagrammatically. In Fig. 67, *1*, the bases of the leaves are represented in the usual way, so as to show their relation to each other and to the central axis. The lowest leaf is indicated by the figure *1*, the highest by *6*. It is easily seen that the leaves lie in five longitudinal planes ('orthostichies'), and that, by proceeding from leaf *1* to the next, one passes round two-fifths of the circumference of the axis. Looked at sideways, as shown in Fig. 67, *2*, one sees that the leaves may be united by a spiral line (the so-called 'fundamental spiral') and that

Fig. 66. Growing point of *Hippuris vulgaris*. After SACHS (Vorlesungen über Pflanzenphysiologie, 1st ed., Fig. 307).

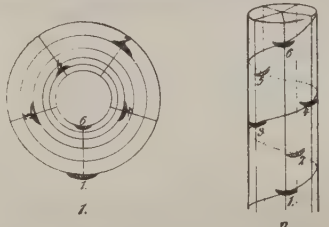


Fig. 67. Diagram of a two-fifths spiral. *1*, in ground plan; *2*, seen laterally.

leaf *6* stands exactly above *1*, *7* above *2*, and soon. This very common arrangement is described as a 'two-fifths spiral' and from this description it will be readily understood what is meant by a one-third, three-eighths, or a five-thirteenths spiral respectively. In simple arrangements, such as the one-third or two-fifths spiral, the numbers indicate for the most part the order of development of the members. It is possible even in such complicated cases as, for example, the succession of the flowers in the inflorescence of the sunflower, to number the lateral members in accordance with certain geometrical rules, and from the numbers to construct a 'fundamental spiral.' In the latter case, however, this has no significance, since we are not entitled to assume that the development of the branches takes place in the order in which the numbers follow each other. The formation of the lateral members frequently takes place more rapidly on one side of the growing point than on the other, and the successive appearance of the young branches is not indicated at all by the position of the member preceding it in the numerical series but arising far from it. The position of the new member depends, for the most part, far more on its immediate neighbours, and hence 'oblique lines' (parastichies) are determinable, of which there are often in *Helianthus* fifty-five in one direction and eighty-nine in the other, so long as growth is regular. Their regularity disappears, however, if the space relationships at the growing point alter, and if the relation between the diameter of the lateral axes and that of the growing point varies (compare SCHWENDENER, 1878; HOFMEISTER, 1868).

It is not possible for us to go further into the question of the arrangement

of lateral branches; on that subject we must refer to the special literature concerned (BRAVAIS, 1837-9; A. BRAUN, 1831; HOFMEISTER, 1868; SCHWENDENER, 1878; GOEBEL, 1898), and merely point out that the general distribution of the lateral members in the examples selected above is uniform on all sides, so that we have before us a *radially* arranged growing point. As an example of a *bilateral* growing point we may take that of *Caulerpa holmesiana*, a unicellular alga (Fig. 68). The lateral buds arise in this case on two sides only, 180° apart, while the sides above and below, parallel with the plane of the drawing, show no outgrowths. If we turn the body round through an angle of 90° we obtain an appearance like that shown at Fig. 68, *II*, where only the bases of the lateral members are indicated. If we turn a radial growing point

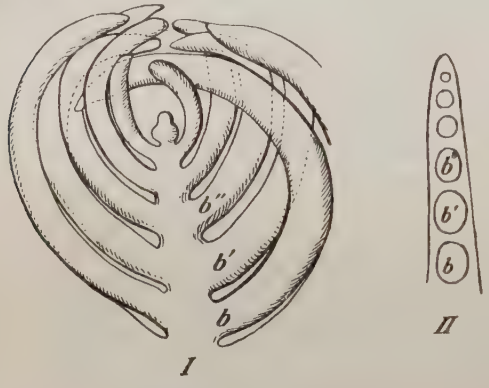


Fig. 68. Growing point of *Caulerpa holmesiana*. *I*, looked at on face; *II*, looked at laterally. After REINKE (1899).

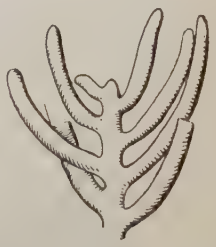


Fig. 69. Growing point of a frond of *Caulerpa obscura*. After REINKE (1899).

through 90°, or, in fact, through any angle, the appearance remains unaltered. In Fig. 68 the lateral axes are arranged in pairs, the members of each pair arising at the same level, and we term such an arrangement 'pinnate'; in so far it corresponds to the whorled type of radial growing point. If, on the other hand, the lateral branches be arranged in two *alternate* rows as in Fig. 69, we have to do with the spiral type and may term it a one-half spiral. Looked at from this point of view bilateral symmetry is merely a special case of radial. *Dorsiventral symmetry* is fundamentally distinct from either of the preceding types (compare GOEBEL, 1880). As an example we may select the inflorescence of *Vicia cracca*, where the whole of the flowers arise on *one* side only (Fig. 70, *1*), while the other side has no outgrowths at all (Fig. 70, *2*). We do not distinguish in this case two *flanks* but a *front* and *back*. The arrangement of the lateral organs individually is of the greatest interest. They are arranged in regular parastichies as on a radial growing point and we could number the organs in this case also were it not that they early assume a unilateral arrangement. We see, however, that unilateral development does not preclude an essentially spiral taxis, confirming the conclusion already come to that the 'fundamental spiral' has no significance of any importance.

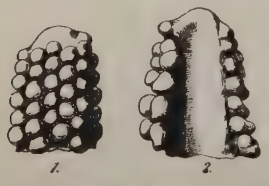


Fig. 70. Inflorescence of *Vicia cracca*. *1*, Front view; *2*, back view. After GOEBEL (1880, Pl. X, Figs. 19 and 20).

We must now consider the *form* of the growing point. In the examples hitherto given it takes the form of a slender paraboloid, or, as it is generally termed, though less accurately, a cone. Very frequently, however, it has the shape of a flattened cone or disc. We meet with such forms not infrequently in

the floral regions of the higher plants, and transitions may be obtained between such forms and the depressed growing point, where somewhat older parts grow round the real 'apex' so as to form a kind of crater, while the younger organs develop progressively downwards on its inner wall (Fig. 71). It may be noted in this relation that this form of growing point may occur not only owing to depression during its development from the rudiment up to its definitely completed state (e.g. many flowers), but that the depression may be induced by supplemen-

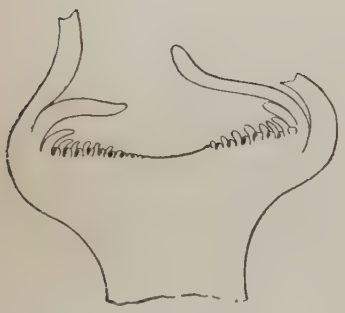


Fig. 71. Longitudinal section through the capitulum of a sunflower. The central, somewhat depressed, growing point shows as yet no rudiments of flowers. Slightly enlarged.

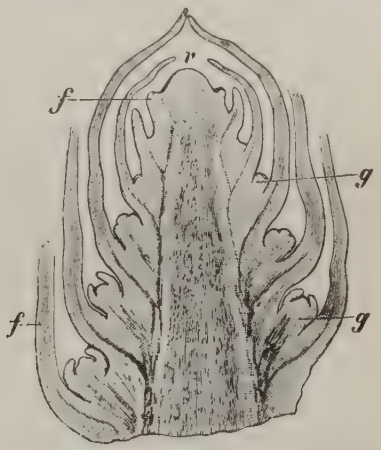


Fig. 72. Longitudinal section of the bud of a Phanerogam. *v*, growing point; *f*, leaf rudiments; *g*, axillary bud (× 10). From the Bonn Textbook.

tary modifications. In the latter case, the depressed situation of the growing point protects this very delicate part from possible injuries from the environment. Such protective arrangements are attained, however, in other ways. In the formation of *buds*, for example, the lateral members grow more rapidly than the apex of the growing point, curving over in such a way as to enclose it. Fig. 72 will give an idea of the appearance of such a bud in longitudinal section. In other ways also, e. g. by *inrolling* of lateral members, a protective arrangement is not infrequently acquired by *dorsiventral* growing points (Fig. 73).

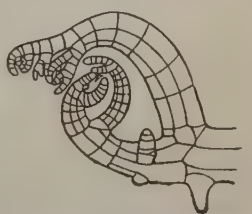


Fig. 73. *Herposiphonia repens*. *v*, growing point. After GOEBEL (1880).

We know now that the duty of the growing point is, while continually elongating its own axis, to give rise also to lateral axes. The same relations in general obtain on these lateral branches as on the chief axis. They have each at least one growing point, originally a process from the chief growing point; it elongates into an axis of secondary rank and eventually gives rise in turn to lateral axes. The lateral branches of the first order, however, do not all behave alike, and even in many Algae one can distinguish two types: those which behave in all respects like the main axis, *long* shoots, and those whose growing point after a short time loses its activity, the *short* shoots. The distinction is not, however, of much importance and intermediate conditions are not infrequent.

There are quite special relationships in the highest plants, and especially among the Phanerogams, which demand our attention. The shoot bears on its axial region, the stem, special lateral appendages which we term *leaves*

One might well conceive of these organs as derived phylogenetically from short shoots, but in reality they exhibit many important points of difference from them. The point which is of the greatest interest to us at present is the difference in the mode of growth. The leaves have in general a very limited capacity for growth, for in a short time they become fully developed, their growing points as such disappear, that is to say, become transformed into permanent tissue. Similarly growth in short shoots is, as we have learned, very limited, but the growing points are often still retained, and can, if the appropriate stimulus be applied, give origin once more to new members. A short shoot may very often change into a long shoot, whilst the alteration of a leaf into a shoot, whether long or short, is impossible. This is certainly the rule, but the rule may have exceptions. Thus, as a matter of fact, we have been for long acquainted with cases among ferns and other plants as well where apical growth continues in the leaves for many years. We know again of many long shoots in which growth is arrested owing to the complete transformation of the growing point. As an example may be taken the case of a shoot ending in a thorn, or flowers where the growing point is generally transformed into an ovary. The difference quoted is not enough to serve as a distinction between a short shoot and a leaf, but there are others, only one of which need be mentioned here, viz. their relative positions on the axis.

There are quite a number of plants which form only one axis on which no lateral members save leaves are produced, e. g. *Isoetes*, many ferns and palms. In a second series may be placed the numerous Coniferae which develop lateral branches only. The majority of the higher plants, however, form both lateral buds and leaves, and between these members perfectly definite space relations subsist. The bud is formed in the upper angle of the leaf insertion, in the so-called leaf axil (Fig. 72, *g*), and is termed in consequence an *axillary bud*. Its point of origin is sometimes more towards the base of the leaf, sometimes more towards the side of the stem ; it appears sometimes at the same moment as the leaf rudiment, sometimes considerably later.

These recognized relations between the leaf and the axillary bud may serve to distinguish these organs from each other, but they are applicable only to radial (and bilateral) shoots. If the growing point be dorsiventral, on the other hand, the leaves arise on the upper side, while the lateral branches arise on the flanks and the roots on the under surface, at some distance, it is true, from the growing point.

The lateral branches in turn may be again branched. The shoots of secondary, tertiary, &c., rank maintain the same relations we have described as characteristic of those of primary rank. It is otherwise in the case of leaves. The *leaf-blade*, the part of special interest to us, is very frequently flat and dorsiventral, i.e, with clearly marked upper and under surfaces Such a surface may be simple or it may be branched. Branching in many cases (Palmaceae, many Aroidaceae) may result from rupture of an originally simple leaf, but usually the lateral branches are predetermined from the beginning. Occasionally dichotomous branching occurs (ferns and many Dicotyledons, e. g. *Utricularia*, *Ceratophyllum*, and many species of *Drosera*) ; for the most part, however, the branching is *lateral* and commences, in the simplest cases (the only ones we are considering at present), either from the base towards the apex (Umbelliferae, Leguminosae) or in the reverse direction from the apex towards the base (*Rosa*, *Potentilla*), or, finally, in the middle, progressing towards either end (*Achillea millefolium*). Each branch arising in this way may again branch in a similar manner. All branches arise on the *flanks* of the mother axis and the individual leaflets lie in this way more or less *in one plane*. The fact that the leaves develop for the most part in a bud, that is to say, in a confined space surrounded by older leaves, necessitates many deviations from the flattened

form. This flattened appearance is generally developed after the opening of the bud and the bud usually exhibits an exceedingly complicated arrangement of its individual parts (prefoliation).

A consideration of the branching of the stem and leaf should naturally be followed by that of the branching of the root. There are, however, reasons why a discussion of this subject should be preceded by a study of the cellular structure of the growing point. The varied methods of formation of members which we have hitherto studied occur as we have already pointed out (compare p. 273) not only in the higher multicellular plants but also in unicellular forms. This is especially the case in the polymorphous genus *Caulerpa* (Siphonaceae), which in its habit resembles one of the creeping forms of the higher plants. Its dorsiventral growing point produces a horizontally growing stem, giving off leaves from its upper side and roots from below, and occasionally forming lateral branches on the sides, and yet the entire plant, many centimetres or even decimetres in length, consists of a *single* cell. The complete similarity between the growing point of this unicellular plant and that of a multicellular type, proves most clearly that cell division cannot have the importance which has for so long been ascribed to it. Nevertheless the subject is naturally of interest in itself and we must devote a sentence or two to its consideration. We shall put on one side the simple growing points which go to form an unbranched plant-body (cell filament, cell surface, or cell body) and glance only at the branched forms, confining our attention further to those which exhibit *lateral* branching.

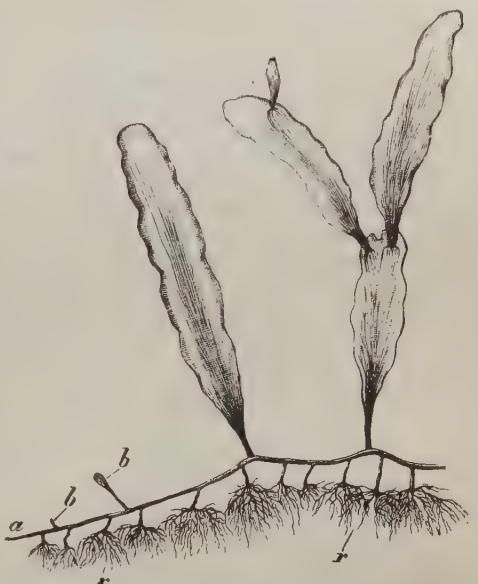


Fig. 64. *Caulerpa prolifera*. *a*, growing point; *b*, leaves; *r*, roots. From the Bonn Textbook.

In the simplest case the growing point consists of a single terminal cell, the apical cell. This cell (*s* in Fig. 75) determines, for example, in *Stypocaulon*, the extension of the chief axis, on which arise alternately to right and left the lateral processes which are the rudiments of lateral branches. They are scarcely formed before they are cut off by a concavo-convex wall (*1*, Fig. 75) while the apex goes on growing. When they have reached a considerable length they divide into two cells by a transverse wall. The distal one retains the characters of an apical cell, and will after a time develop a lateral branch to the left, while the lower cell divides by successive walls into a row of cells and thus becomes a cell body.

Fig. 75. *Stypocaulon scoparium*. *s*, the apical cell; *1*, *2*, *3*, *4*, successively older lateral branches.

This is an extreme case. Generally, the apical cell itself does not give rise to lateral branches directly, but only indirectly, from the segments cut off from it. The growing point is further not limited to the apical cell but includes in addition a number of cells, and posteriorly merges gradually into cells which

are fully developed. The form of the apical cell is, however, still identical with, or at least very like, that of *Stypocaulon*. At the ends of the stems of mosses and ferns we meet with another form of apical cell, distinguished by being two- or three-sided. In the latter case it has the form of a tetrahedron or a three-sided pyramid with its curved base facing outwards. Cell divisions take place parallel to the three surfaces which face inwards and follow each other in definite order, so that the individual segments which are cut off from the three-sided pyramid are arranged in three rows behind the apical cell. Very soon, however, further division-planes divide these up into a large number of cells.

The stem-apex of the Phanerogam is more complicated still. Here we find no predominant apical cell to which all the other cells may be referred ; the apex consists, on the other hand, of a group of many cells. Fig. 76 shows the conical growing point of an aquatic plant as seen in longitudinal section. An axial cellular strand (*pl*) stretches backwards and is surrounded by four enveloping layers of cells (*pr*), whose outlines almost converge into five confocal parabolas. These parabolas are traversed by a number of parabolas cutting them at right angles, which have the same focus and the same axis, but which are

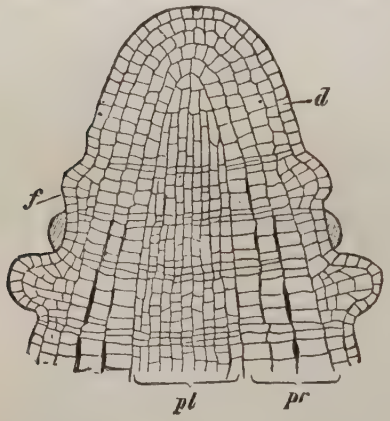


Fig. 76. Longitudinal section through the growing point of *Hippuris vulgaris*. *f*, leaf primordia. From the Bonn Textbook.

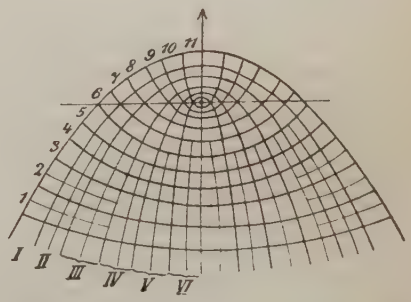


Fig. 77. Diagram of the arrangement of cell-layers in the growing point. After SACHS (1878).

laid out in the opposite direction. The orthogonal trajectories of the first-mentioned parabolas are not so apparent because they are often interrupted ; still they are seen in SACHS'S schematic figure (Fig. 77) as complete curves. We will confine any further remarks we have to make to this figure, thereby definitely affirming that the small differences between the diagram and what occurs in nature are of no consequence so far as the correctness of our exposition is concerned. In the diagrammatic longitudinal section we note *rows of cells* which appear like bent cell filaments ; those which run almost parallel with the outer surface, and indicated in the figure by the Roman numerals *I-VI* we term periclines, those crossing them, *1–11*, we term anticlines (SACHS, 1878). In both periclines and anticlines the same phenomenon occurs, viz. that the series with lower numbers are flatter, while those with higher numbers are always more bent, until finally the highest numbers of all are curved round the apex and the two arches touch each other in the line of the axis. In order to give some conception of the probable mode of growth of a growing point of this kind, Fig. 78 shows on the left of the median line the upper portion only of Fig. 77 so far as the anticlines *7-11* are concerned, while to the right the same five anticlines are represented at a later stage of development. It will be seen from this diagram that

each individual anticlinal cell row has become twice as broad as it was, and has become divided by new anticlinal walls into *two* layers of cells. The periclinal rows have elongated considerably, but have become obviously broader at their *bases* only, where further divisions have also taken place. This result has been arrived at on the special assumption that *all* the anticlines, *7–11*, have been growing at about the same rate, and this assumption *may* be true in nature of a certain part of the growing point. It cannot, however, be generally true ; for a maximum or a minimum growth may equally well occur at the *apex* while towards the base a *gradual* change ensues. Since, unfortunately, the anticlinal rows are not so obvious in nature as in the diagram, we are on the whole ignorant of the exact nature of the growth divisions taking place in them. We know more about the periclinal divisions ; their gradually increasing transverse extension may be deduced off-hand from Fig. 76. In the illustration the growth division has not as yet proceeded so far as to result in periclinal division. If the growing point be less conical, periclinal divisions soon appear beneath the apex. It is only on the outermost layer, the future epidermis (*d*, Fig. 76), that these periclinal divisions cease in all cases. In the growing points of mosses and ferns the distribution of intensity of growth can be made out much

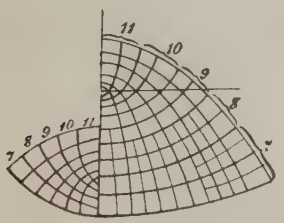


Fig. 78. Diagram of a growing point in longitudinal section. The right hand half represents a later stage in the development of the left hand half.

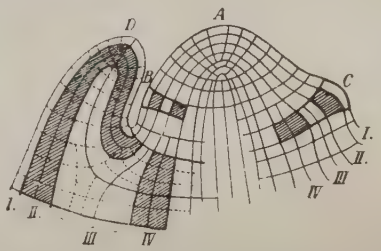


Fig. 79. Diagram of the growing point *A*, with successively older leaf rudiments, *B*, *C*, *D*. After SACHS (1879).

more accurately than in Phanerogams owing to the very regular divisions resulting from the activity of the apical cell (compare WESTERMAIER, 1881).

The rule followed at the growing point as to the direction of the new cell-walls is that which we have already become acquainted with. The new walls appear throughout as ' minimum areas ', and in very many cases the new walls arise at right angles to those already in existence. We cannot follow out this subject further however.

Leaf formation always occurs below the apex of the growing point. In the mosses this is seen especially clearly because in that group each segment cell gives rise to a leaf. The leaves are thus laid down in a one-third spiral, but the segment cells of the axis which give rise to them at once undergo peculiar changes in form (CORRENS, 1899; SECKT, 1901), which bring about torsions and so lead to great complexity in the leaf arrangement. The outer wall of the segment cell forming a leaf rudiment bulges outwards, and from this bulging is next developed the two-sided apical cell destined to give rise to the leaf. In the ferns relationships as intimate as these between the stem segments and the leaves can no longer be distinguished, and the origin of the latter takes place as in the Phanerogams, save that in the ferns the apical cell of a leaf may still be distinguished, while in the Phanerogams it cannot. The leaf formation of the Phanerogams may be made out from Fig. 76, but more clearly, perhaps, from SACHS's diagram (Fig. 79). In the diagram three successively older leaves *B*, *C*, *D*, are represented beneath the apex *A*. It shows that the leaf arises from

one or more adjacent anticlinal cell rows which by their active growth create a prominent swelling on the outer surface of the growing point. The cells of the periclinal row, *IV*, take scarcely any part in the formation of this swelling, while on the other hand, pericline *III* shows extreme activity both in growth and in cell formation, and the swelling so formed is covered by the periclinal layers *II* and *I*. Layer *II* has become double, but the outermost layer (*I*) remains, as elsewhere, unilamellar. The leaf rudiment grows progressively along with the growing point of the stem from which it arose; later on,

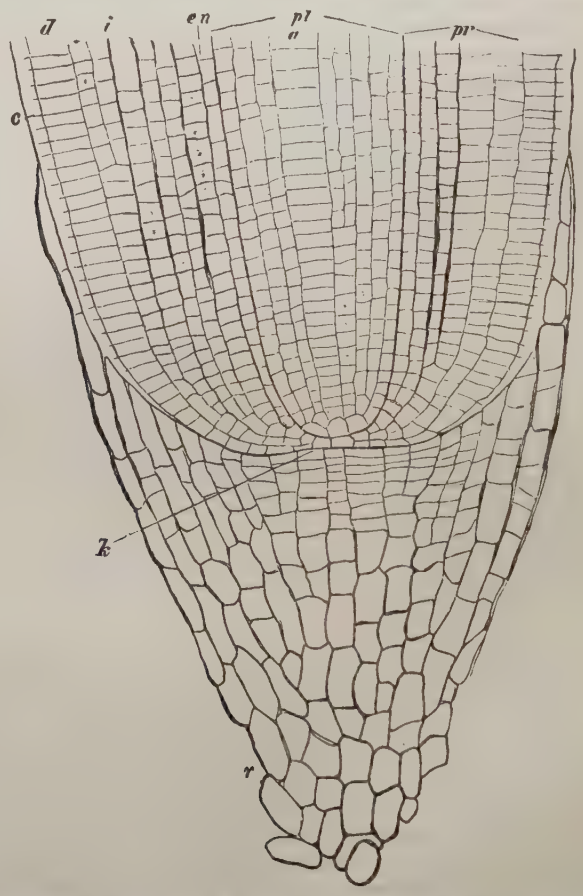


Fig. 80. Median longitudinal section of the root apex of *Hordeum vulgare*. *k*, initial cells of the root-cap; *r*, cells of the cap about to be thrown off; *pl*, central cylinder; *en*, endodermis; *i*, intercellular spaces; *d*, epidermis; *c*, thickened external wall of the epidermis. From STRASBURGER (Das botanische Prakticum, 4th ed., Jena, 1902).

when growth becomes more marked perpendicularly to the plane of the drawing, the flattened dorsiventral shape of the typical leaf is assumed. The lateral buds arise in the same way as the primary growing point of the leaf. The manner in which the young leaf is attached to the growing point of the stem is worthy of notice. At first the leaf rudiment has approximately the form of a hemisphere, whose surface in contact with the stem is nearly circular in outline (elliptical or rhomboidal). Frequently, however, this projection proceeds to grow in two directions, encircling the growing point, so that finally it forms a ring-like wall, whose greatest height lies medianly or oppo-

site the point at which it first arose. Thus the stem comes to be enclosed by successive leaf sheaths.

The lateral branches arise practically in the same way as the leaves, so that we need not discuss them further. The root, however, from its special peculiarities claims our attention. The growing point of the root is always intercalary and forms new and different structures in two directions. The apex of the root is always covered by a rootcap which protects the delicate growing region. The rootcap, so far as its function is concerned, may be compared with the other protective adaptations to which we drew attention in speaking of the growing point of the stem. It consists of simple parenchymatous tissue which is especially well developed at the extreme apex of the root, but also encloses the *sides* of the root for some distance. Its cells are short lived, but they are constantly renewed by the growing point itself. In spite of this renewal the rootcap does not increase in size because the older cells die off in front and are cast off as new ones are formed behind. On the other hand, the root itself exhibits continued increase in length at the apex owing to the activity of this same growing point. In detail there are important differences in structure among root apices, but all that concerns us is the general principle on which they are formed, so that we may confine ourselves to *one* example, e. g. the longitudinal section of the root-apex of *Hordeum* shown at Fig. 80. We see that the growing point is constructed in a manner similar to that of the stem, especially as regards the regularity of the *periclinal* divisions. The formative layers of the rootcap are seen at *k*. (As regards the remaining features see the explanation beneath the figure.) If, as in ferns, an apical cell occurs, the cutting off of segment cells follows entirely the rule which governs segmentation of the apical cell of the stem, save that segments are cut off on the fourth or *outer* side of the pyramid also, and from these the cap is formed.

The difference between root and stem comes out especially in the branching. The root produces no leaves and no buds but lateral roots only, which are quite like the main root. Hence branching in the root is extremely uniform. The point of origin of the lateral root branches is, however, quite unique. 'Root buds', comparable with stem buds, do not exist. The terminal part of the root is quite free from lateral roots for a considerable distance, and when they do appear, some way back from the growing point, they burst out from *within*, through tissues already full grown; in other words, roots arise *endogenously*, while leaves and buds arise *exogenously*. At first sight it might appear as though the lateral roots could not be derived from the growing point of the main root, as though in short we had here an exception to the rule which we have established for the stem, viz. that *every new growing point is a portion of a previously existing one*. When, however, we examine a transverse section of a fully-developed root we notice a layer of cells lying within the parenchymatous cortex separated off by the 'endodermis', and which, under the name of 'pericycle', surrounds the central vascular system. This pericycle may be traced right up to the growing point, and its cells have the peculiarity of retaining for long the characters of embryonic tissue. When the whole of the surrounding cells have been transformed into permanent tissue they remain still capable of division. The pericycle is in short a residuum of the growing point and it is from it that the lateral roots arise. Their origin from the pericycle is illustrated in Fig. 81.

After the young root has been laid down it grows out to the exterior by dissolving and mechanically destroying the cortical tissue before it. We shall see in our next lecture wherein lies the biological significance of the late formation of lateral roots; at present it is sufficient to note the fact that in spite of their late formation we can still refer their origin to the growing point of the chief root, and that the rule enunciated above, which we may, with SACHS, term

briefly the ' continuity of embryonic substance ' applies to the root as well as to the stem. [As to this embryonic substance, compare NOLL, 1903.] Every growing point is a portion of an older one; all arise in the long run from the growing points of the embryo, and these in turn come from the egg-cell. The egg-cell, however, is itself formed from the growing point of the parent plant.

We must not forget to note that in addition to this *normal* development of new growing points there is also another method. One example of this phenomenon may be referred to. The much-cultivated Begonias are multiplied by laying isolated leaves on wet sand. New buds then develop from certain epidermal cells (HANSEN, 1881), which in turn speedily form roots and become independent. As we have seen, the leaf generally ceases to grow at a very early stage and the epidermal cell of a fully-developed leaf is a type of a permanent tissue element. So long as the conditions are normal, such a cell will not show any visible signs of capacity for growth. Since, notwithstanding, this cell becomes, under certain conditions, a growing point it proves that the capacity for becoming such was latent only, and became actual on the application of a stimulus. Such cases of *adventitious* origin of growing points are remarkably widespread. They teach us that the difference between fully-developed and embryonic cells is a quantitative and not a qualitative one, and the correctness of this view will become more evident when we study the subject of cambium (Lecture XXIII).

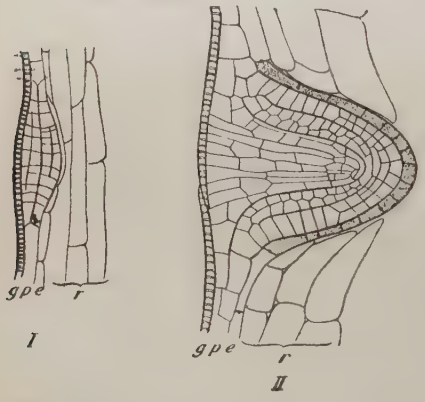


Fig. 81. Portion of a longitudinal section through the main root of *Reseda*. *g*, vessels; *p*, pericycle; *e*, endodermis; *r*, cortex. After VAN TIEGHEM (Ann. sc. nat. 1898, ser. 7, vol. 8.)

Turning back once more to simple plants and plant parts, we see that in many cases the most vigorous growth occurs at the growing point, and that just behind it fully-developed regions are met with. We have already (p. 261) studied such cases in considering the apical growth of fungus cells and root-hairs, but similar phenomena are to be seen in more complicated growing points which form lateral branches, e. g. in *Caulerpa*, where also growth rapidly ceases behind the apex (compare REINKE, 1899, p. 61). The same appearance is shown by multicellular Algae. In *Stypocaulon*, for example, the whole growth takes place in the apical cell, and cell division follows in completely developed segments of the apical cell. Doubtless, detailed research would establish the same results in many other plants. Nor is it remarkable that it should be so, for there are plenty of plants in which nothing else is possible than the localization of growth in embryonic cells since they consist of such cells only.

In contrast to this case stands another type represented especially by the higher plants. Growth at the apex of the growing point is very restricted, and the plants attain their proper length only by extension of cells which are situated at a greater or less distance from the apex of the growing point. Thus we must follow SACHS in distinguishing a *primary* growth period, when the members are laid down, a *secondary* when they are elongated, and usually also a *tertiary* when their internal differentiation is completed. [BERTHOLD (1904) attempts to distinguish *six* growth regions: (1) the initial region (the apex of the growing point); (2) the region of morphological subdivision; (3) the region of anatomical differentiation; (4) the region of elongation; (5) the region of

extension ; (6) the region of completion. The first two regions only correspond to our 'growing point', the remaining regions will be dealt with in the next lecture.] Obviously, the growth periods pass gradually into each other, still they differ sufficiently to justify a special treatment of the second and third in the next lecture, just as we have dealt with the more important phenomena presented by the first in the present lecture. A detailed discussion of the growing point will be found in SACHS (1882), and GOEBEL (1884).

Bibliography to Lecture XXII.

[BERTHOLD. 1904. Untersuchungen z. Physiologie d. pflanzlichen Organisation. Leipzig, 2, 185.]
BRAUN, A. 1831. Nova acta acad. Leop. 15, I, 199.
BRAVAIS. 1837. Annal. Sc. nat. II, 7, 42.
BRAVAIS. 1839. Ibid., II, 12, 5.
CORRENS. 1899. Festschrift f. Schwendener. Berlin.
GOEBEL. 1880. Arb. d. bot. Inst. Würzburg, 2, 353.
GOEBEL. 1884. Vgl. Entwicklungsgesch. der Pflanzenorgane (Schenk's Handbuch der Botanik, 3). Breslau.
GOEBEL. 1898. Organographie, 1, 61. Jena.
HANSEN. 1881. Abh. d. Senkenbergschen Gesell. 12.
HOFMEISTER. 1868. Allgem. Morphologie d. Gewächse. Leipzig.
NOLL. 1896. Sitzungsber. Niederrhein. Gesell. f. Natur- u. Heilkunde, 3. Febr. 1896.
[NOLL. 1903. Biol. Centrbl. 23, 281.]
REINKE. 1899. Ueber *Caulerpa* (Wiss. Meeresunters. Kiel, N. F. 5, 1).
SACHS, J. 1878 and 1879. Arb. d. bot. Inst. Würzburg, 2, 46 and 185. Gesam. Abhdl. 2, 1067, &c.
SACHS, J. 1882. Vorles. über Pflanzenphysiologie, p. 939. Leipzig.
SCHWENDENER. 1878. Mechan. Theorie d. Blattstellungen. Leipzig.
SECKT. 1901. Bot. Centrbl. Beihefte, 10.
WESTERMAIER. 1881. Jahrb. f. wiss. Bot. 12, 439.

LECTURE XXIII

ELONGATION AND INTERNAL DIFFERENTIATION

WE have learned to recognize in the continued activity of the growing point an essential difference between the plant and the animal. In the latter the primordia of all the organs are laid down in the embryo, and growth continues for long afterwards, often for many years, during which the *enlargement* of these embryonic primordia unto the fully adult condition takes place, but a *fresh formation* of new organs from a persistent embryonic substance occurs only in plants. We might say, indeed, that a normal plant is *never full grown*, but consists of fully grown parts coupled with parts having a capacity for further development. This difference between an animal and a plant is, however, not so thoroughgoing as one might at first sight suppose.

Growth, as we have seen, may be restricted to the growing point, so that the axis attains its prescribed length and thickness at quite a short distance behind the active apex, and the rudiments of the lateral organs are placed at the same intervals apart that they maintain later on. In other cases apical growth is only feebly developed, and the organs which arise from the growing point frequently exhibit, at a certain distance from it, conspicuous increase both in length and in thickness. It is with these phenomena of *elongation* that we have to deal in this lecture.

We will commence by studying a member of the family of liverworts (Askenasy, 1874). In *Pellia epiphylla* the fertilized egg-cell gives rise after several months to a sporogonium, of which only the median region, the seta, is of interest to us at present. The young seta consists of numerous embryonic cells, but even after several months growth is only 2 mm. long. When the spores begin to ripen, however, it begins to grow rapidly, reaching a length of 80 mm. in three or four days, thus elevating the capsule into the air. The cells of the seta are at first filled with protoplasm and contain abundant starch, but after this great elongation has occurred, the starch is found to have been completely used up (having been employed in the manufacture of new cell-walls), and the protoplasm now forms merely a thin layer in the interior of each cell, which is occupied chiefly by large vacuoles. It is especially interesting to note that no cell division occurs during the elongation. This elongation does not take place equally and uniformly throughout the entire length of the seta, but by differentiation of a zone of maximum growth, which, however, does not remain constantly in one place.

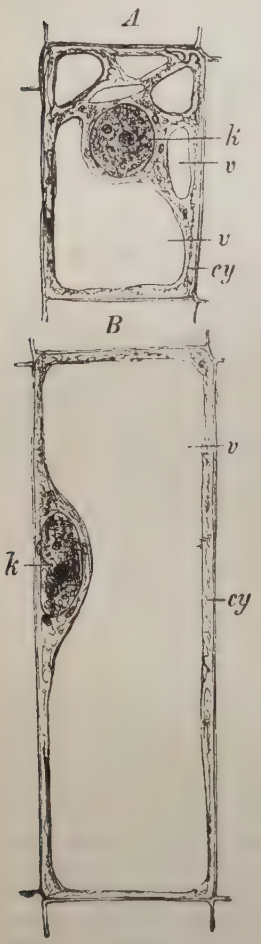


Fig. 82. Elongation of cells accompanied by increase in the size of the vacuoles (× 500). From the Bonn Textbook.

Similar growth elongations are known to occur among other lowly-organized plants such as certain of the larger Fungi and many Algae, such as the unicellular Siphonaceae. These appearances are met with, however, especially among the higher plants, and to these we may now direct our attention. The chief features of this elongation are as follows :—

1. Growth at the very beginning is accompanied by cell division, but later on this division ceases, and the cells elongate considerably.

2. The increase (Fig. 82) in the volume of the cells is by no means equalled by the increase in the amount of the protoplasm, hence the vacuoles become larger and larger till they reach a quite remarkable size, apparently by absorption of water. In this way growth may be effected rapidly and at little cost of material.

3. Growth is not uniform, but varies in amount in a manner still to be described.

Various methods are employed for measuring growth ; we will describe those first which aim at determining the total growth in length of an organ. For rough measurements an ordinary scale is sufficient ; for more accurate measurements, especially when these have to be taken successively at short intervals, we employ a reading microscope. In dealing with vertically growing structures such as roots and shoots the tube of the microscope must be placed horizontally, and for this purpose Pfeffer's horizontal microscope will be found very appropriate. In addition to the optical method of magnifying the amount of growth we may employ mechanical means, e. g. a lever. A very simple form of ' auxanometer ' is that used by Sachs, which consists of arc and pointer (Fig. 83). A light wire frame carries a small pulley (*r*) and a long pointer (*z*) playing on a scale (*q*). If a fine silk thread be attached to the apex of the plant and then carried over the pulley and kept stretched by a small

weight, every elongation exhibited by the plant will cause a movement of the pulley, which will be exaggerated by the pointer. This instrument does very well for lecture demonstration. The more elaborate auxanometers employed in scientific research are constructed on the same principle, but are arranged so as to record the amount of elongation automatically. In Fig. 84 we have again a pulley, serving the same purpose as in SACHS's apparatus; it is, however, connected with a *larger* pulley by means of which the movement is still further exaggerated. Over the larger pulley runs a silk thread bearing at one end a writing style, which registers the growth movements of the plant on a blackened revolving cylinder. Registering auxanometers of this sort have been designed by WIESNER (1876), BARANETZKY (1879), and PFEFFER (1887).

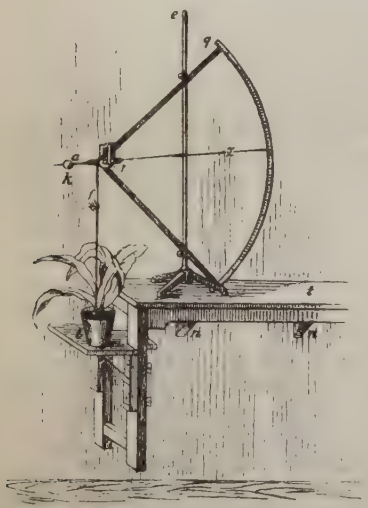


Fig. 83. Simple auxanometer. After DETMER (Smaller Practical Botany, Jena, 1903).

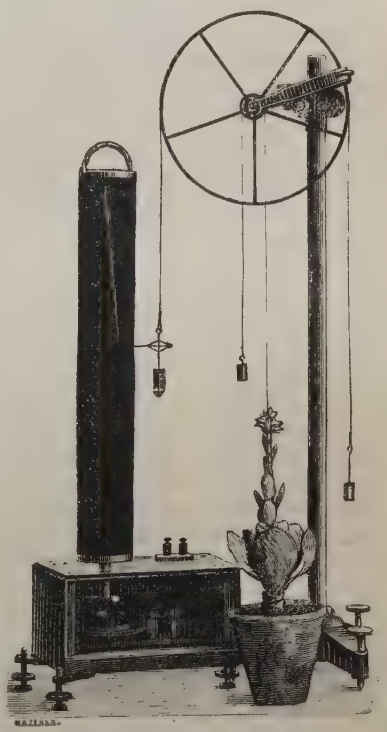


Fig. 84. PFEFFER'S auxanometer, as manufactured by ALBRECHT of Tübingen.

If we have to deal, not with the total growth of the plant, but with the *distribution* of growth in it, and the amount of growth in different *zones*, we must measure, macro- or micro-scopically, segments mapped out by natural or artificial marks (usually made with Indian ink), and observe the distances these marks are apart at successive intervals of time.

We will now endeavour to make ourselves acquainted with the characteristic features of growth in the root and shoot; but we must take care to see that in growth calculations all external factors, more especially temperature, are kept as nearly as possible constant. We will begin with a study of growth in the *root*, and in order to do this most conveniently we will cultivate the plant in water. Should we desire to study its growth in natural surroundings we employ boxes of sheet zinc filled with soil, but with one side replaced by a sloping plate of glass. We allow the root to grow backwards along this plate and observe it from without. SACHS (1873) marked off a zone just behind the growing point on the main root of a seedling of *Vicia faba* by two fine lines of

Indian ink 1 mm. apart, and found that in the following days this zone increased in length in accordance with the following numbers :—

Days	1	2	3	4	5	6	7	8
Increase in mm.	1·8	3·7	17·5	16·5	17·0	14·5	7·0	0

The rate of growth at first is slow and then rapid; the maximum rate is maintained for a certain time, later on it decreases and finally ceases altogether. This phenomenon, which has been observed in all cases where growth has been measured, has been termed by SACHS (1872) the '*grand period of growth*'.

We will now mark out with Indian ink on a root not one transverse zone only, but several, beginning at the growing point, and passing backwards, each being 1 mm. apart. If we calculate the increase in relative length of these zones on the following day we shall find that the increase is different in each zone, but that the differences are undoubtedly subject to certain laws. A few examples will make this clear. In the following table the several zones marked off from the growing points are indicated by the numerals I, II, III, &c., and the growth-increase during 22-24 hours is indicated in mm. (means of several measurements) :—

	XII.	XI.	X.	IX.	VIII.	VII.	VI.	V.	IV.	III.	II.	I.	Total.
Vicia faba (SACHS, 1873)	0	0	0·2	0·6	0·7	0·8	2·0	3·5	6·5	8·0	2·5	1·0	25·8
Vicia faba (POPOVICI, 1900)	0·25	0·35	0·5	1·0	1·25	1·5	2·5	4·0	6·0	12·0	7·0	1·0	37·35
Phaseolus (POPOVICI, 1900)	0	0·25	0·25	0·35	0·6	1·0	1·5	3·0	5·0	7·0	16·0	1·0	35·95
Peas (SACHS, 1873)	0	0	0	0	0	0·3	0·5	1·5	3·0	5·5	4·5	0·5	15·8

It will be seen from these different examples that only a few of the zones marked out on the root are growing in length, and that the grand period begins at I and ends at XII. In order to obtain a better conception of the periodic changes in rate of growth in the individual zones we will attempt to give a graphic representation of the example (*Vicia faba*) first cited. We will represent the time in hours by abscissae, and the lengths of the zones at the beginning of the experiment and after twenty-two hours by ordinates, and endeavour to draw curves indicating roughly the successive increments of growth (Fig. 85), assuming that growth as a whole is uniform, and that the length of the growing zone remains 10 mm. in length. This mode of representation shows very clearly that the upper zones, after a very brief interval, have completed their growth, while the lower ones, usually after several hours, begin to elongate. We see that a certain zone, the third in the figure, has attained the greatest length, and we also see that the position of maximum growth must shift as time goes on, always approaching the apex. To make this clearer still we will express Fig. 85 by a series of measurements as in the following table. The zones, each 1 mm. long, have increased by lengths corresponding to the successive numbers indicated (in mm.) :—

Hours: 0	3	6	9	12	15	18	21
X. 1·0	1·2 growth completed						
IX. 1·0	1·5	„	„				
VIII. 1·0	*1·8*	„	„				
VII. 1·0	*1·8*	2·0 growth completed					
VI. 1·0	1·6	*2·8*	„	„			
V. 1·0	1·2	*2·8*	*4·2*	4·6 growth completed			
IV. 1·0	1·1	1·4	3·2	*5·0*	*6·4* growth completed		
III. 1·0	1·0	1·2	1·4	2·2	4·4	*6·8*	*8·6*
II. 1·0	1·0	1·0	1·0	1·2	1·2	1·8	3·0
I. 1·0	1·0	1·0	1·0	1·0	1·0	1·2	1·6

We see from this that the *maximum* growth is reached after three hours in the zones VIII and VII, after six hours in VI and V, and steadily advances thereafter until, after eighteen to twenty-one hours, it occurs in zone III. If we were to allow more time to elapse between each two measurements we should

find the maximum elongation finally taking place in zone I. That the graphic representation figured at Fig. 85 agrees in this important point of the *shifting of the zone of maximum growth* will be seen by comparing it with SACHS's measurements (1873), some of which we may quote :—

Vicia faba.

	Increase in mm.									
	X.	IX.	VIII.	VII.	VI.	V.	IV.	III.	II.	I.
Increase in the first 6 hours.	0·1	0·1	0·5	*1·0*	*1·0*	0·5	0·4	0·3	0	0
,, in the next 17 hours	0·1	0·2	0·3	0·5	1·5	2·5	*4·1*	3·7	2·0	1·0
,, after 24 hours . .	0	0	0	0·4	0·5	1·5	3·0	*5·6*	4·5	1·8
,, after 48 hours . .	0	0	0	0·4	0·5	1·5	3·0	6·6	*15·0*	5·0
,, after 72 hours . .	0	0	0	0·4	0·5	1·5	3·0	6·6	17·0	*23·0*

It is obvious, however, that this forward march of the zone of maximum growth towards the apex is *apparent* only ; for our curves show in the clearest possible way that the maximum zone is always situated at approximately the *same distance from the apex*, and if we carry out our observations at short intervals and always *mark afresh* each time, we shall find that this will be clearly proved by the *measurements* also. We thus see that to allow longer time to elapse between each pair of observations in determining the region of maximum growth would lead to serious error.

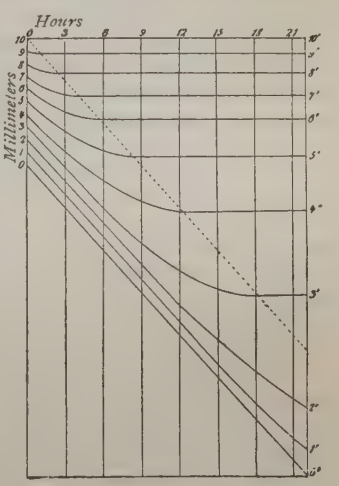


Fig. 85. For explanation see Text.

By way of summary we may say : *The growing region of the root is limited to a few millimetres behind the growing point. In the course of this growing region each individual transverse zone passes through a grand period, those zones which are nearest to the apex are at the beginning, those farthest away from it at the end of their grand periods.*

The *shortness* of the growing region in the root is a matter of great importance to it. The root in its passage into the soil has to overcome great opposition, and we may compare it (SACHS, 1873, 424), to a nail driven into a plank of wood. As in the case of the nail so in the root there is a danger of bending : the shorter the growing point the more safely it enters the soil. If we contemplate this entry of the root into the soil, the pointed form of the growing point covered by its cap is explained, and we further appreciate the reason why the lateral roots develop first at some distance behind the apex, and from parts which have long before attained their maximum growth and have now become quiescent. If the new roots arose from the growing point itself they would add to the difficulties the root has in entering the soil, or they would have to form a kind of bud—just as, in fact, we see stem buds, e. g. of seedlings, boring through the soil.

Roots which do not live in soil, especially the long aerial roots of lianes and epiphytes, exhibit a much longer growth zone, as SACHS (1873) long ago pointed out, and as WENT (1895) has more recently confirmed. Thus WENT found a growing zone 40 mm. long in *Philodendron*. These aerial are, in fact, comparable with shoots, growth in which we will study by and by.

Let us now inquire what the total growth resulting from the addition of the several increases of the individual zones amounts to, as evidenced by the forward development of the root apex in space. We assumed above that growth was uniform, and, acting on that assumption, we have represented the undermost of our curves (Fig. 85) by a straight line. ASKENASY has, indeed, shown that

the roots of maize grow at a relatively uniform rate, for with approximately half-hourly observations he obtained the following increases in growth calculated up to one hour :—

	Growth increments, calculated by micrometer ($1 = \frac{1}{21}$ mm.).								
Hours	1	2	3	4	5	6	7	8	9
Root No. 1	34·0	27·0	30·0	29·5	36·0	35·0	38·0	31·0	33·5
Root No. 2	32·5	34·5	37·9	34·5	33·0	33·6	33·0	—	—

These growth increments may be, therefore, considered as relatively uniform, while in other bodies for the most part much greater variations have been observed, variations the causes of which are not known, and which we may describe as 'spasmodic growth variations'. If the observation of a root be carried on not merely for several hours but for days or weeks, we find that the total growth also shows a ' grand period ' (PEDERSEN, 1874).

The growing point of the *shoot* is, as we have seen, enclosed by leaves growing more rapidly than the apex itself and thus forming a 'bud'. In many annuals and perennials, and also in some trees, we find at the growing point, during the whole summer, the rudiments of new leaves and the parts of the axis relating to them ; they at once become transformed from the rudimentary condition into the adult by elongation. This is not the case with the majority of trees. In these cases during summer and autumn all the parts of the bud within the leaves, which act as bud-scales, undergo slow embryonic growth, and these parts become unfolded in the following year. In this case embryonic growth and growth in length are sharply separated. Thus in many Coniferae one sees in autumn, after removal of the bud-scales, a green cone several millimetres long covered with small spirally arranged outgrowths. This is the rudiment of an entire shoot which will elongate in the following year in the course of a few weeks. In other trees the same features are seen, but the buds for the most part are not so easily examined as in the case of the spruce. The elongation, however, may take place in a few *days* (beech).

The cases in which growth consists merely in the *elongation* of parts *laid down* in the *previous* year are the simplest to understand, and we will begin with them. Two types have to be distinguished (ROTHERT, 1894). The whole bud may behave uniformly and grow approximately equally in all its parts, or exhibit a distinction into nodes which grow but little and internodes which grow vigorously. The unsegmented bud axis of the spruce, which may serve as a type of the unsegmented shoot, becomes uniformly elongated in springtime throughout its entire length, and may become five times as long as it was during the winter, attaining in this way about one-tenth of its ultimate length. In the course of further extension a zone of maximum growth appears, which lies at first at the base of the shoot, but passes gradually nearer and nearer to the apex. Exact measurements demonstrate the fact that each individual zone of a spruce-shoot passes, during its elongation, through a grand period.

Let us now compare the bud of *Fritillaria* with that of the spruce. This bud is divided into nodes and internodes, but *one* only of the many internodes elongates actively, and a grand period may be demonstrated in its case with the greatest readiness. The following table gives relative elongations of this internode (SACHS, 1872, 129) :—

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
mm.	2·0	5·2	6·1	6·8	9·3	*13·4*	12·2	8·5	10·6	10·3	6·3	4·7	5·8	4·4	3·8	2·0	1·2	0·7	0

If, finally, all the internodes of a bud become elongated (as, for example, in the case of the horse-chestnut) we obtain just as many growth zones as there are internodes, separated by nodes which grow slightly or not at all. Each

individual internode obviously goes through its own grand period, although we know but little as to the distribution of growth in it.

The case becomes more complicated if the shoot exhibits not merely growth extensions of parts already laid down, but if it continually adds new parts to those already present in the growing point, and if these also begin to elongate. If the shoot be undivided into nodes and internodes as, for example, in *Asparagus*, *Linum*, &c., growth may in general be considered as equivalent to that seen in the root; a single growth zone only is developed and in it a single maximum. The only difference between such a case and the root is that the growth zone is *much longer*. We are acquainted with shoots which have growth zones 10 cm. or even 40–50 cm. in length, but in these cases the region of maximum growth lies much further back from the apex than it does in the root.

As an example of a shoot with obvious segmentation and with a continuously growing apex, we may select *Chara* or *Nitella*. These highly-organized Algae increase by means of a terminal apical cell. Each segment of this apical cell divides into two cells; the upper cell is biconcave, and after several subdivisions becomes a node, the lower biconvex cell remains undivided and becomes an internode. The nodes retain approximately their original length, in *Nitella* about 0·02 mm. (ASKENASY, 1878); the internode, on the contrary, becomes stretched often as much as 2,000 times its original length. If we correlate the length relationships of successive internodes on an actively growing shoot we obtain the following values (ASKENASY, 1878) :—

Internode	1	2	3	4	5	6	7	8
Length in mm.	0·02	0·07	0·16	0·45	3·33	14·0	33·5	35·0

If we make the not improbable assumption that one internode undergoes similar extensions in similar intervals of time, as shown above in the case of successive internodes, then each internode will exhibit the following grand period, where the increments in similar periods of time are indicated in mm. :—

0·05 0·09 0·29 2·88 10·77 19·5 1·5

In fact each individual internode in a segmented shoot passes through a grand period independently. In each, also, a zone of maximum growth may be demonstrated at some definite place, and possibly this shifts from the base towards the apex (or in the reverse direction from the apex to the base), in the same way as we have seen it do in the shoot of *Picea*. It not infrequently happens that the region of the stem, where the zone of maximum growth occurs last of all, exhibits not a simple stretching of the cells merely, but both *cell formation* and *cell elongation*, lasting for a long time. In every individual internode a portion of the primitive growing point remains, and this goes on acting as an *intercalary growing zone*. In fact there is no line of demarcation between localized extension and an intercalary growing zone.

The question now arises, what is the total amount of growth resulting from the activity of several independent growth zones? It is known that often 3–4 or, in other cases, as many as fifty internodes are elongating at the same time. The sum of their activities *may* give a single uniform curve of growth *not* differing from that of the single internode of *Fritillaria* given above, or it may be entirely different (ROTHERT, 1894). If only a few internodes be concerned in the elongation, it might come about that a younger internode might start growing after the older one had entirely or nearly ceased to grow, and thus we should have a periodic rise and fall of the growth curve, that is to say 'spasmodic variations' such as we have previously drawn attention to. Such variations are, generally speaking, to be found almost everywhere; they owe their origin, however, doubtless *not* to the cause just mentioned only. The

spasmodic variations in the growth of *Bambusa* are very remarkable (KRAUS, 1895) as may be seen from the following curve (Fig. 86).

We can distinguish, finally, in the *leaf* during its embryonic growth, as a rule, two regions, a proximal and a distal. From the distal region is developed the blade, from the proximal part arises either a leaf-sheath or merely a flattened point of attachment to the stem, which may become enlarged into a well-differentiated structure, 'the pulvinus'. In accordance with the area of the leaf attachment to the stem, in so far must it elongate with the stem, and thus we see the growing stems of Coniferae densely covered with pulvini, as is specially evident in the spruce itself. In all cases where the leaves are thus developed close together, the leaf-bases must accompany the stem in its elongation as in the Coniferae. It is true one very often sees nothing of them externally, and distinct pulvini may be entirely absent. All the same a careful investigation will show generally the existence of such where a free stem-surface between the leaf rudiments does not exist from the outset at the growing point.

Between the leaf-base and the blade there appears frequently in the growing

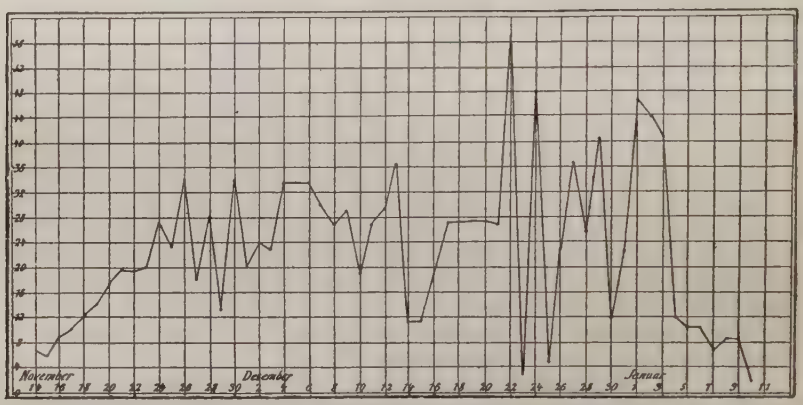


Fig. 86. Daily growth increments (in cm.) in the stem of *Bambusa*, measured at Buitenzorg from Nov. 13, 1893, to Jan. 10, 1894. After KRAUS (1895, Pl. 20).

leaf a very distinct region, the leaf-stalk, which arises generally by intercalary growth in a zone of tissue of minimum extent between the leaf-base and the blade, but only after the blade has progressed considerably in its development. We learned previously that at the beginning the blade also shows apical growth. Only in a few cases does this apical growth continue for long, generally it ceases long before the rudiments of all the parts are complete, or at least before their elongation commences. Among ferns, *Gleichenia* and *Lygodium* are known to possess leaves with apical growing points which remain active for years, and even among our ordinary ferns it may happen that new pinnae are developed at the growing point when the basal ones have already opened out. Similar cases occur in Phanerogams, as we learn from the researches of RACIBORSKI (1900), who found that in certain of the Meliaceae (*Guarea*, *Chisocheton*), the power of producing new pinnae at the apex of the leaf was long retained. According to SONNTAG (1887), the leaf of *Guarea* possesses only a brief apical growth, during which a limited number of pinnae are laid down, which expand partly in the first, partly in the second vegetative period. The supposed likeness to ferns thus breaks down, or to speak more accurately, it is limited to a slow and purely *acropetal expansion*, which occurs also elsewhere.

Frequently we meet with another type of leaf-expansion where the *apex*

at once passes into a state of rest. This is the case in many lianes, where specially formed apices fulfilling particular functions ('forerunner-tips,' RACIBORSKI, 1900) are produced long before the rest of the lamina is completed. The elongation is basipetal also in the long leaves of Monocotyledons, owing to the development of an intercalary growing zone at their bases. The distribution of growth in this case is illustrated by the following numbers, which represent the fortnightly increments in zones, each 2.5 mm. long, marked off on the leaf of the onion (STEBLER, 1878) :—

	Leaf-sheath.		Leaf-base.				Leaf-apex.	
Zone	I.	II.	III.	IV.	V.	VI.	VII.	VIII.	IX.
Increase	7·9	26·4	25·1	48·1	30·1	19·0	16·7	10·4	1·4

Intercalary growth zones are of frequent occurrence in leaves; but it is impossible for us to enter into a discussion of the effect such intercalary growth zones have on the formation of the leaf; reference must be made to the morphological literature, and more especially to GOEBEL (1898-1901).

It was noted earlier that leaves during their embryonic growth assumed special formations, which were explicable partly mechanically, partly biologically. If, taking a simple case, the leaf, owing to excessive growth on the under side, protects its growing point by bending over on itself, it is obvious that this curvature must be again compensated for during elongation by increased growth on the upper side. Unequal growth in length of this kind is to be found not only in cases where it is necessary to compensate for previous curvatures or foldings, but it occurs also by no means infrequently in uncurved rudiments, transforming them from a straight embryonic form to a permanently curved adult form. The physiologist sees, more often, indeed, than he desires, how the root-apex or the tip of the shoot pushes itself forward, not in a straight line but in a curve, for such curvatures, known as nutations, resulting from small irregularities in growth of the different sides, frequently interrupt experimental work to a serious degree. We shall take another opportunity of referring to such cases.

Only a few examples of specially rapid growth need be quoted, for the measurements which have been noted vary extremely. The following table gives the maximum rate of growth *per minute* for a few plants :—

Dictyophora	(MÖLLER, 1895)	5	mm.
Stamens of Gramineae	(ASKENASY, 1879)	1·8	,,
Bambusa	(KRAUS, 1895)	0·4	,,
Coprinus	(BREFELD, 1877)	0·225	,,
Botrytis	(REINHARDT, 1892)	0·034	,,

There are cases known where it is possible to watch the organ actually growing without employing a microscope. These observations have, however, no scientific value, since the *actual rate of growth*, i. e. the increase in *unit of length* per *unit of time* cannot be expressed. The growing region in *Bambusa* is very long (several centimetres); in *Botrytis* it is only 0·02 mm. in length, for although the former shows ten times as great an increase as the latter per minute, still its rate of growth is much less. To work out the rate of growth we must use *percentages*. The following table gives the increase per cent. of the growing zone per minute (BÜCHNER, 1901) :—

Pollen-tube of *Impatiens hawkeri*	220	per cent.
,, ,, *balsamina*	100	,,
Hypha of *Mucor stolonifer*	118	,,
Botrytis	83	,,
Stamens of Gramineae	60	,,
Shoot of *Bambusa*	1·27	,,
,, *Bryonia*	0·58	,,

We may also calculate the *time* necessary for the attainment of a certain increase, e. g. for doubling the length of the organ :—

Botrytis	1 min.
Bacteria	20–30 ,,
Grass stamen	2–3 ,,
Root of *Vicia faba*	about 180 ,,

If we know the *duration of growth* we may from the rate of growth and the extent of the growing zone calculate the definite amount of elongation a plant part undergoes. According to the variations in these factors the size of the plant is determined, and it is, as every one knows, dependent also on external factors in manifold ways, and yet in each case it is specifically different. *Draba verna* in the course of its vegetative period attains the dimensions of a few centimetres, *Ricinus* or *Helianthus* must be measured in metres; *Calluna vulgaris*, after ten years, still remains a small shrub, but a *Eucalyptus* tree reaches the height of Strassburg Cathedral (compare p. 62). A definite size is as much a specific characteristic of an organism as leaf-arrangement is; the entire organization of a plant is co-ordinated with the attainment of a certain size. This is a point which SACHS (1893) has demonstrated most clearly, showing what an impossible monstrosity would result were a *Marchantia* enlarged fifty-fold, or diminished to a like extent.

We have as yet limited ourselves to a consideration of the *longitudinal* extension of the parts mapped out at the growing point. [BERTHOLD (1904) has, as already remarked on p. 284, advanced 'stretching' as a characteristic phase of growth in addition to elongation. He understands by that term the 'inflation' which many parenchymatous cells of the leaf, the root, or the stem-cortex undergo after the whole organ has reached its maximum growth in length. Whether this 'stretching' is identical or not with the primary increase in thickness about to be described it is impossible for us to judge.] Every microscopic investigation, however, demonstrates the fact that increase in thickness also takes place. The diameter of the fully-formed root or stem is greater, and often markedly so, than that of the region just behind the growing point. This is demonstrated by Fig. 76, which shows an increase in the diameter downwards in the periclinal cell rows. Growth in thickness has been much less carefully studied than growth in length, still all the essential features which we have learned to recognize in growth in length are also found here. In the first place we can establish the existence of a 'grand period'. On account of anatomical relations we distinguish 'primary' and 'secondary' growth in thickness. *Primary* growth in thickness is universally distributed, and consists in the increase in size of *all* cells, which at first divide, but which cease to do so later on. Not infrequently primary growth in thickness begins vigorously just after growth in length ceases, and FRANK (1892) has established the fact that an internode of the sunflower which has reached its greatest length may increase in diameter until it has become nearly five times its original size. Many plant-organs which exhibit considerable dimensions in the transverse direction, e. g. fruits, tubers, &c., must attain their permanent form by such primary growth. In certain groups, especially in Dicotyledons and Gymnosperms, we find another mode of increasing in thickness which we may term *secondary*, and by which a continuous growth is effected in stem and root for many, even hundreds, of years. The difference between primary and secondary increase in thickness does not lie in *duration* however, for in palms (and also tree-ferns) primary increase in length goes on for many years; the characteristic feature of secondary increase in thickness is the existence of a special growing layer, an intercalary zone known as cambium. Cambium, in so far as it lies within the vascular bundle, is formed from a tissue which remains over, after the formation of the bundle, between the xylem and the phloem regions, which does

not become transformed into permanent tissue, but retains its embryonic characters. This cambium is a remnant of the primary growing point. If it alone be present then the bundle only increases in thickness ; but for the most part there arises also an interfascicular cambium, i. e. certain cells of the medullary rays, having all the characters of permanent tissue elements, revert to the embryonic condition and form cambium arcs uniting themselves to the already existing cambiums on either side. In this way arises in a cylindrical organ, a complete circular (in transverse section) intercalary growing layer which produces new tissue actively and continuously. Fascicular and interfascicular cambiums possess the same capacities, so that it is immaterial whether the origin is directly or indirectly from the growing point. Ordinary parenchymatous-cells are simply intermediate stages between embryonic and permanent tissue-elements, which retain for a long time, frequently all their lives, to a certain degree, a capacity for renewal of growth, although they do not always exercise it. Although we may make a sharp distinction between primary and secondary growth in thickness, we do not do so with regard to growth in length ; more careful consideration shows, however, that the intercalary growing regions, such as those at the bases of leaves of Monocotyledons or in many internodes, have as much right to be termed secondary growth areas as cambium has. We may, therefore, speak perfectly legitimately of secondary growth in length.

Before we leave the consideration of growth in length we must study certain peculiar phenomena concerned with the relationships of growth in length and in thickness. If longitudinal growth be rapid, a diminution in diameter may take place, and vice versa. A diminution in diameter, although quite insignificant, occurs, according to Askenasy (1879), in the stamens of Gramineae, which in a quarter of an hour may increase their length fourfold by absorption of water. The converse process occurs much more frequently ; it was found by Berthold (1882) in *Antithamnion*, and is of common occurrence in roots. In the latter (De Vries, 1880 ; Rimbach, 1897), immediately after vigorous elongation, an increase in thickness occurs which causes a reduction in length of as much as 10–70 per cent. This reduction is due to a certain alteration in form taking place in *some* but not *all* cells. Owing to the activity of these cells, an activity not as yet sufficiently well understood, other tissues, such as those of the cortex and of the vascular system, which are unable to contract, are thrown into folds. This contraction in the root is of very great importance. Its effect is to draw down more and more towards the soil the leafy regions of many ' rosette plants ', in spite of the continued elongation of the axis by growth ; it causes and regulates the entry of many tubers and bulbs into the soil to a definite depth, and finally strengthens the hold the plant has on the soil, because tense roots render the plant as a whole more stable than slack ones.

Following Sachs, we have distinguished three periods of growth ; during the first of these the different members are *laid down* on a certain plan, during the second their *absolute* and *relative size* is determined, during the third (which we have now to deal with) the internal anatomy is developed. These three periods, as already noted, cannot be sharply marked off from each other, more especially in the case of the third, which often begins before the first has come to an end.

When the growing point is multicellular, its cells are generally full of protoplasm ; each possesses a large nucleus and shows no vacuoles. The growing point in such plants as the Siphonaceae or Mucorinae, which consists merely of a part of one cell, also shows, for the most part, a dense aggregation of protoplasm in that region. We might, therefore, conclude that abundance of protoplasm is one of the most general characteristics of an embryonic cell and closely bound up with its specific function. A number of facts, however, tend to show that this conception is incorrect. Thus Noll (1902) found that the

activity of the growing point of the Siphonaceae was quite independent of the presence there of a large amount of protoplasm. [Further, NOLL (1903) has also shown that the protoplasm in the apex of the growing point does not differ from the remainder, that it overflows into it and is supplied from it.] He also showed that in numberless lower plants every cell is in an 'embryonic' condition, and yet that the protoplasm is present there in only moderate quantity. The ordinary condition of the cells found at the growing point must be explained otherwise. PFEFFER (Phys. II, p. 7) points out that the abundance of protoplasm may be accounted for by assuming that it is intended to permit of rapid growth in length by the taking in of water, without any further construction of protoplasm.

From the cells of the growing point are derived all the permanent tissue-elements in the higher plant, no matter how varied they be in appearance and in function. Their differentiation takes place at different times in the several organs. While, for example, the definite structure may be mapped out, though not completed, in an internode still undergoing elongation, anatomical differentiation takes place in a root at a later stage, often long after elongation has ceased. Certain elements, such, for example, as sclerotic cells, which have no longer the power of growth, may expand after the completion of their legitimate length, while, on the other hand, vessels generally push ahead of all other elements. Their early appearance is obviously necessary because the demand made on water by the growing points can only be supplied by a continuous water channel.

To trace the transition of embryonic cells into permanent tissue-elements in detail would necessitate a study of the fundamentals of plant anatomy; we must limit ourselves, therefore, to a consideration of principles only and refer for details to the special literature on the subject, especially HABERLANDT (1896).

Let us first look at the alterations in the general *outline* of the cells. As they are approximately isodiametric at the growing point, they must become pulled out during longitudinal growth unless their original length is reproduced by continuous transverse division. The *relative* length, that is to say, the relation of length to diameter, may be increased by several longitudinal divisions. Very frequently there is a tendency on the part of cells to *round* themselves off; in this way walls meeting each other at an angle of 90° get displaced in such a way that now three walls meet together at one point at an angle of 120°. At the corners or angles also, owing to increased stretching, a splitting of the middle lamella often takes place, in consequence of which intercellular air-spaces appear, communicating with each other; these spaces are of extreme importance in relation to gaseous exchange. Possibly all these rounding-off processes may be explained in the first instance by osmotic pressure in the interior of the cell, in regard to which the cell-wall behaves passively. But active local growth of the membrane is also an important factor in form differentiation. Just as from epidermal, so occasionally from internal cells bordering on intercellular spaces outgrowths (hairs) may arise. The vessels may become filled with 'thyloses', ingrowths from neighbouring cells which become pressed against each other, and so the vessels may become blocked up by a luxuriant cellular growth entering through clefts formed in the course of development (e. g. between separated sclerotic rings). Local surface growth may appear also in individual cells in compact tissues, and these cells may force their way between neighbouring elements, splitting their middle lamellae and sliding over their cell-walls. This kind of growth has been termed 'sliding growth', and appears to be of much more general occurrence than was at first imagined. When no individual cell grows more rapidly than its neighbour, but the *whole tissue* shows equally vigorous surface growth, the resulting form is attained without

breaking the continuity, and so long as this continuity is maintained, the difference in growth may be accounted for by mere *tension*.

These tensions have been termed *tissue tensions*, and they deserve a word of explanation at this point. If we extract, by means of a cork-borer, the medulla of a young internode of the sunflower, we may easily observe that the isolated medulla increases in length by a certain percentage, while the peripheral region shortens also to a certain extent. In the uninjured stem the cortical region is in a state of extension while the medulla is in a state of compression. If we split the stem longitudinally the two halves bend outwards like bows, so that the cortex and the medulla assume their proper lengths, the former contracting, the latter expanding. If we strip off a ring of cortex from a branch and sever it at one point and then attempt to put it back again over the wood, we shall find that the ring is too short; it has contracted tangentially. Transverse tension, therefore, exists as well as longitudinal. These phenomena of tissue-tension have been studied with great care, because it was expected that conclusions as to various physiological phenomena might be obtained from them. These expectations have not been fulfilled, and hence the brevity of our reference to the subject. When we come to the consideration of the phenomena of movement we will take an opportunity of again referring to the matter.

After this digression let us return to the subject of tissue-differentiation. In addition to the form of the cell, the peculiarities of its membrane are of importance. Just as from a *chemical* point of view we may distinguish a whole series of alterations in the cell-wall, so from the *physical* standpoint the cell-membrane may exhibit varied modifications. Lastly, there are the *morphological* differences occasioned by the degree to which the membrane becomes generally or partially thickened.

Thirdly, there are all the *cell-contents* to be taken into account. The presence or absence of vacuoles, nuclei, chloroplasts, leucoplasts, starch-grains, and other constituents, are all characters of the several types of cell. In many cells contents are absent altogether, that is, the living contents are replaced by water and air; nevertheless these constituent cells may also carry out important functions in the plant.

Lastly, we must note that certain elements, when fully formed, may unite with other neighbouring elements of similar form by complete or partial absorption of their transverse walls. Such *cell-fusions* stand in marked contrast to single cells, but this contrast is neutralized by the fact that the independence of individual cells, notwithstanding the sliding growth previously referred to, is by no means complete. For all living cells are connected to each other by delicate strands of protoplasm which pierce the cell-membranes, and on the basis of this discovery we may affirm that in the complex plant, whose body is broken up by numberless cell-walls, only one protoplasmic body is to be found, just as in *Caulerpa* or *Mucor*.

Bibliography to Lecture XXIII.

Askenasy. 1874. Bot. Ztg. 32, 237.
Askenasy. 1878. Verb. naturw. Verein Heidelberg, 2, 1.
Askenasy. 1879. Ibid. 2.
Askenasy. 1890. Ber. d. bot. Gesell. 8, 61.
Baranetzky. 1879. Mém. Acad. Pétersbourg, VII, 27, No. 2.
Berthold. 1882. Jahrb. f. wiss. Bot. 13, 607.
[Berthold. 1904. Phys. d. pflz. Organisation. Leipzig. 2, 146.]
Brefeld. 1877. Unters. aus d. Gesamtgebiete d. Mycologie, 3, 61.
Büchner. 1901. Zuwachsgrösse u. Wachstumsgeschwindigkeiten bei Pflanzen. Leipzig.

FRANK. 1892. Lehrbuch d. Botanik. Leipzig. 1, 376.
GOEBEL. 1898–1901. Organographie d. Pflanzen. Jena.
HABERLANDT. 1896. Physiol. Pflanzenanatomie, 2nd ed. Leipzig.
KRAUS, Gr. 1895. Annales Jardin Buitenzorg, 12, 196.
MÖLLER, A. 1895. Schimper's bot. Mitt. aus den Tropen. Jena. 7, 119.
NOLL. 1902. Sitzungsber. niederrhein. Gesell. f. Natur- u. Heilkunde.
[NOLL. 1903. Biol. Centrbl. 23, 281.]
PEDERSEN. 1874. Arb. d. bot. Inst. Würzburg, 1, 569.
PFEFFER. 1887. Bot. Ztg. 45, 29.
POPOVICI. 1900. Bot. Centrbl. 81, 35.
RACIBORSKI. 1900. Flora, 87, 17.
REINHARDT. 1892. Jahrb. f. wiss. Bot. 23, 479.
RIMBACH. 1897. Fünfstück's Beitr. z. wiss. Bot. 2, 1.
ROTHERT. 1894. Cohn's Beitr. z. Biologie, 7, 1.
SACHS. 1872. Arb. d. bot. Inst. Würzburg, 1, 99.
SACHS. 1873. Ibid. 1, 385.
SACHS. 1893. Flora, 77, 49.
SONNTAG. 1887. Jahrb. f. wiss. Bot. 18, 236.
STEBLER. 1878. Jahrb. f. wiss. Bot. 11, 47.
DE VRIES. 1880. Landw. Jahrb. 9, 37.
WENT. 1895. Annales Jardin Buitenzorg, 12, 1.
WIESNER. 1876. Flora, 59, 467.

LECTURE XXIV

EXTERNAL CAUSES OF GROWTH AND FORMATION. I

THE form of the plant is determined by a large number of factors, which we may divide into two groups; internal factors, i. e. those originating within the organism itself, and external factors, i. e. those emanating from the outer world. In nature, external and internal factors always co-operate, and none of the plant activities can be manifested under the influence of *one* set of factors only. Still, for the purpose of investigation and description, we must, as far as possible, keep the two series distinct. Among external factors—the only ones we will consider at present—we may distinguish the purely chemico-physical influence of the inorganic surroundings from the complex influences resulting from association with other organisms. We have already dealt with the dependence of certain functions (e. g. respiration, assimilation, &c.) on external factors, and have now to study the influence of these factors on *growth*; we will not limit ourselves, however, to this phenomenon only, but inquire into the action of the outer world on *life as a whole.*

It is not our intention to give a complete catalogue of all the known effects produced by every individual factor; we must content ourselves with a few examples without going into every influence which affects the *whole period of growth,* for sometimes embryonic growth, sometimes elongation or internal differentiation, will be found to claim our chief attention.

We may note in general that all external factors (PFEFFER, Phys. II, 85) operate either directly or indirectly. *Direct* effects, where the external factor provides the *energy* for the resulting phenomenon, are remarkably rare, while *indirect* influences may be recognized almost everywhere. The external world acts as a *stimulus* on the plant, and in co-operation with the special capacities possessed by the plant, it brings about certain results. We may recognize amongst stimuli a first series of factors which we shall term *formal conditions*; they are absolutely *essential,* if any development is to take place, they are the essential *conditions of vitality.* Then again we have also stimuli which are *non-essential,* but which, all the same, produce conspicuous results when applied to the plant. All stimuli may be regarded first as either *accelerating* or *retarding*

the rate of growth, and in this respect their influence is ephemeral; or, secondly, they guide growth and form into definite channels (*formative stimuli*), in so far as they influence either only the number and size, or also the position, symmetry, direction, or polarity of organs. The results produced are in individual cases either *quantitative* only or, in more complex cases, *qualitative* as well.

Let us begin with the consideration of the influence of *ether* vibrations, and of these we need consider here only *heat* and *light*, for electricity plays no part in determining growth and form in plants. At the very outset we become conscious of the fact that growth, and indeed all the vital processes in the plant, take place only within certain limits of *temperature*, and the phenomena of everyday life prove to us that these limits are most varied amongst different plants—a fact which is confirmed by scientific research. Just as in other functions we discover that there are three cardinal points in temperature for growth, a minimum, a maximum, and an intermediate optimum, the data with regard to which we may take from PFEFFER (Phys. II). Arctic Algae appear to be able to grow at temperatures under 0° C., but the minimum temperature for most fresh-water Algae lies about 0° C., or slightly higher. Among higher plants seeds of *Triticum vulgare* and *Sinapis* begin to germinate just above 0°, while *Phaseolus* commences its development at 9°, *Cucumis sativus* at about 16°, the bacillus of tuberculosis at 30°, and the thermophilous Bacteria at temperatures even higher still. The *maximum* temperature for many marine Algae is especially low; there are, however, no data on record as to the maxima which Algae inhabiting arctic seas can tolerate. The very low maximum of 16° is recorded for *Hydrurus* (a fresh-water alga), while that of the majority of land plants lies somewhat between 30° and 45°; it is only in the case of succulents that growth may take place at a temperature of from 50° to 52° C. (compare p. 44). On the other hand, thermophilous Bacteria can flourish in media which, owing to fermentative processes, exhibit a maximum temperature of 75°, and certain Algae can endure temperatures even higher than that, e.g. in the neighbourhood of natural hot springs. LÖWENSTEIN (1903, Ber. d. bot. Gesell. 21, 317) finds, however, that the Algae in the Karlsbad springs cannot endure a temperature above 52° C. In general terms it may be said that in the case of plants inhabiting cold regions both cardinal points of temperature are *low*, while in the case of those accustomed to warm surroundings, and also of parasites in warm-blooded animals, these points stand *high*; the range of temperature between these maxima is very considerable, ranging as it does from 16° in the case of *Hydrurus*, between 30° to 40° for the majority of plants, up to 50° or even more in many Cactaceae. As may easily be understood, the requirements of the plant as regards heat, indicated by the position of the minimum and maximum cardinal points, is a factor of fundamental importance in determining the distribution of plants on the earth's surface. The position of these points is by no means fixed, since they may be altered both in higher plants and also, and more especially so, in Bacteria. Thus DIEUDONNÉ (cited by PFEFFER, Phys. II, 91) found that he was able, by gradual acclimatization, to get *Bacillus anthracis* to endure a minimum of 10° instead of 12°–14°, and *Bacillus fluorescens* to withstand a maximum of 41·5°, in place of the normal 35°. It has also been shown that the position of the cardinal points often depends on other factors, such as food, oxygen, light, &c. (compare PFEFFER, Phys. II, 91). In addition to specific differences there are also differences in the case of individual organs and various developmental stages. For example, the flowers of many of our spring plants develop at a much lower temperature than the vegetative organs, which begin to appear after the flowering period is over (*Tussilago*, *Crocus*, cherry, &c.). *Germination* of spores in *Penicillium* takes place between 1·5° and 43° C., the further *growth* of the mycelium between 2·5° and 40° C., and the *formation of spores* only between 3° and 40° C. (WIESNER,

1873). A high temperature appears to be required for the formation of roots in cuttings.

Within these limits, however, the different temperatures are by no means of equal value to the plant. In studying the rate of growth we discover that as the temperature increases growth at first becomes more active, but that later on a higher temperature retards it. If we make a graphic representation of the amount of growth per unit of time in relation to temperature we obtain a curve which at first rises and then falls; the highest point of the curve is known as the growth *optimum*, and this lies sometimes medianly between the minimum and maximum, or sometimes nearer to one than the other. The curve, moreover, has generally not one *principal apex* only, but several secondary ones as well, and these occur, for unknown reasons, very irregularly. A glance at the following estimates, which we owe to KÖPPEN (1870), will show this.

Lengths of the hypocotyl of *Pisum* in 48 hours (average of several measurements).

Temp.	10·4°	14·4°	17·0°	18·0°	21·4°	23·5°	24·2°	25·1°	26·6°	28·4°
Length	5·5	5·0	5·3	8·3	25·5	30·0	45·8	27·8	53·9	23·0
Temp.	28·5°	29·0°	29·9°	30·2°	30·6°	30·9°	31·1°	33·5°	33·6°	36·5°
Length	40·4	24·5	34·6	38·5	40·8	28·6	38·9	23·0	8·0	8·7

If the maximum be exceeded growth gradually ceases, but the life of the plant is at first not necessarily in danger; the organism comes to be in a state known as 'heat rigor'; the condition of being *capable of growth* within the temperatures bounded by the maximum and the minimum we term 'thermotonus'. A temperature 1°–2° in excess of the maximum acts injuriously in a very short time, and is fatal if exposure to it be continued for long. While *Penicillium* can tolerate for many days a temperature 1° C. above the maximum, many Phanerogams exposed to such an ultra-maximum remain alive only for 1–1½ hours (HILBRIG, 1900). The more the maximum is exceeded the more rapidly does death ensue. It is obvious that the absolute degree of temperature sufficient to cause death will show wide variations, since it is closely related to the maximum growth temperature. As examples of such specific differences it may be noted that *Vicia faba* dies at a temperature of about 35° C., *Secale* at 44° (HILBRIG, 1900), and other plants at about 50° C. (SACHS, 1864). That similar variations occur in the case of organs of one and the same plant is shown by LEITGEB'S observation (1886) that all the cells of the leaf of *Galtonia*, save the guard-cells of the stomata, were killed in ten minutes by exposure to a temperature of 59°. Further, there are many resting-stages of the plant, especially the spores of Bacteria, which are insensible in a high degree to temperature, since many in the resting-stage can stand the temperature of boiling water for long though not permanently. All parts of plants which can endure drought are uninjured by high temperatures so long as they are *dry*. Seeds, spores, mosses, and lichens, can often endure 100°–120° in *dry* air. We are quite as ignorant of the causes of death by heat as of the causes of thermotonus; at all events we must not assume, as is so often done, that it is due simply to coagulation of proteid; indeed the fact that death due to heat may occur at quite low temperatures militates against that view.

As in the case of supra-maxima so infra-minima of temperature may retard growth, leading to a condition of 'cold rigor'. While some organisms are killed rapidly by continued cold rigor, or in other words by freezing, others may continue alive for months or even years in that condition. Death from cold takes place in certain tropical plants (MOLISCH, 1897) at temperatures above 0° C., in other cases far below 0° C. In the case of turgid organs a formation of ice must take place within them if the temperature be sufficiently low, and it has been proved (MÜLLER-THURGAU, 1886) that many plants die the very moment the formation of ice takes place. Potatoes, for instance, can remain alive at −2° C. if the formation of ice be prevented, while they die at −1° C. if the ice be allowed

to form. In such cases the formation of ice-crystals must be the cause of death. This is all the more extraordinary seeing that other plants, such as our forest trees and many herbaceous plants which grow during the winter (*Stellaria media*, *Senecio vulgaris*), can endure alternate freezing and thawing many times in succession. Frozen members are, however, at length killed by lowering the temperature still further, and no turgescent cell can tolerate indefinite reduction of temperature. Possibly death is due in many cases only to the withdrawal of water in the formation of ice, a withdrawal which plants can tolerate only to a certain degree. At least the fact is that certain anhydrous organs, such as seeds and spores, are *not* killed by being subjected to the very lowest temperatures, e. g. −200° C. for five days (BROWN and ESCOMBE, 1895), or −250° for a shorter period (THISELTON-DYER, 1899). [MEZ (1905) has shown that many plants do not suffer injury by the withdrawal of water that takes place on the formation of ice, and which always ceases at a temperature of −6° C., but are killed by lower temperatures. In this case death is due to actual cold, not to loss of water in consequence of low temperature.] To go further into the problems which this subject suggests would carry us too far, so that we may refer for further details to the literature on the subject, and especially to PFEFFER'S (Phys. II, §§ 65–68) treatment of the question.

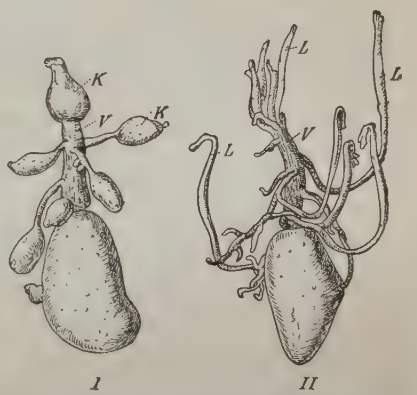


Fig. 87.—Tubers of 'Marjolin' potatoes. *I*, after cultivation for four to five weeks at a temperature of 6–7° C.; *II*, after cultivation for seventeen days at 25° C. The roots are not represented in the drawings. *V*, main shoot, *K*, tubers, *L*, etiolated leafy shoots. After VÖCHTING (Bot. Ztg., 1902, Pl. 3).

We have up to now learned that temperature acts as a stimulus on the thermotonic plant, either accelerating, retarding, or altering the rate of growth, and that these effects are most commonly exhibited during the period of elongation. Notwithstanding differences in the rate of growth, the absolute size and a similar shape may be reached, if only the *duration* of growth alters correspondingly. This is a phenomenon of quite *general* occurrence; plants growing at optimum temperatures do not show differences in appearance from those which have been cultivated at supra- or infra-optima. Changes begin to appear, however, when the limiting temperatures are approached; near the minimum the length of the growing region increases, and shortens near the maximum (POPOVICI, 1900); further, the internodes become shorter if the culture be maintained for long at a low temperature, lengthened duration of growth does not affect the necessary elongation. Temperature thus induces also a *formative* result; such results have been recorded, but they apparently play on the whole only a limited part. VÖCHTING (1902) has recorded a far-reaching effect of alteration of temperature in the case of the variety of potatoes known as 'Marjolin'. At from 6° to 7° C. tubers arise from the main shoot (Fig. 87, *I*), but at 20° C. these are replaced by normal leafy shoots (Fig. 87, *II*). Qualitative differences such as these, however, are only rarely induced by temperature.

When we turn to *light* we are dealing with a factor which obviously must always have a very great influence on the formation of the plant (compare p. 251). The effect of light on growth is *fundamentally* different from that of heat. Many organisms can pass through their entire life-cycle in darkness; in other cases, at least some parts, e.g. roots, can do without light, but *light is generally not a direct condition of growth*. Indirectly it is certainly essential to the existence

of plant life, and indeed of all organisms, since it provides the energy whereby the green plant builds up organic material, on which in turn all organized life on the earth depends. We are not, however, concerned here with this aspect of the case. If we provide for the supply of a sufficient quantity of nutrient materials, then the great majority of plants and plant parts can grow without light. Light is not generally essential to that condition of capacity for growth which we may term phototonus, in analogy with thermotonus; in other words, withdrawal of light does not, as a rule, induce a darkness-rigor in the plant. In many cases which we may describe as darkness-rigor, we are dealing in reality with phenomena which are only *indirectly* dependent on the absence of light (JOST, 1895). Only in a few cases can it be shown that light is an essential formal condition of growth. Thus it has been established that many seeds germinate badly or not at all if they be kept constantly in the dark; *Viscum album* (WIESNER, 1894), *Veronica peregrina* (HEINRICHER, 1899), and *Nicotiana* (RACIBORSKI, 1900) may be cited as conspicuous examples of this. This phenomenon is especially common in the spores of ferns and mosses (BORODIN, 1868; LEITGEB, 1876). That we have not in this instance to deal with an assimilatory action of light and the renewal of the necessary constructive material, but with a specific stimulatory action, is shown by the fact that if tobacco seeds soaked in water be exposed to light for an hour they can germinate in darkness, further, that germination of the spores above mentioned may take place in air from which carbon-dioxide has been excluded, and, finally, that light may be replaced by other stimuli, such as a temperature of 32° C. in the case of fern-spores, and a sugar solution in the case of moss-spores (HEALD, 1898; GOEBEL, 1896).

In such cases as these, which must be considered as exceptions to the rule, we may speak of a *minimum* intensity of light essential to development. There is a definite *maximum*, however, affecting the *generality* of plants, which, when exceeded, first retards growth, and, finally, causes death. The position of the maximum is again specifically very varied. It lies very low in shade-loving plants such as we find abundantly in woods, or, more especially, in the sea. Such plants are killed by direct sunlight. The same is the case with many Bacteria, which are killed by a brief exposure to direct sunlight, or even diffuse light. The light maximum is much lower still for many subterranean organs than it is for shade-loving plants. Thus it is well known that the tubers of the potato bud out readily in the dark, while such development is retarded in diffuse daylight. Darkening has a similarly favourable effect on many, but not all roots, as we shall see later on. Plants which naturally grow in sunny regions are those best adapted to the highest light intensity, and the maximum in their case is reached only when the sunlight is concentrated by means of a lens. The different organs of the plant are not all equally sensitive; thus the chloroplasts are much more easily injured than the rest of the protoplasm.

During the course of development of individual organs the position of the light maximum varies often very considerably. It is only while in the young state that the shoots of the potato are strongly retarded in their growth by light. We meet with very remarkable relationships in the Cactaceae, whose shoots in the long run lose their power of growth as a result of illumination. When put in the dark growth recommences and continues for a time after re-illumination. The growing point may be stimulated to further growth if placed in the dark. Many aquatics behave in a similar manner, e. g. *Elodea*, *Ceratophyllum*, *Myriophyllum*, for MÖBIUS (1895) found that the full-grown internodes of such plants started growing once more when placed in darkness. On the other hand, light exercises a retarding influence on the embryonic growth at the growing point in the Cactaceae and on the growth in length of internodes in the aquatics mentioned.

Just as extreme variations occur in the position of the maximum and minimum of illumination, both in the case of individual plants and individual organs, so also it is impossible to make any general statement as to the position of the optimum.

In nature, all the organs of a plant which are sensitive to light are subject to frequent variations of light intensity, not only owing to the periodic alternations of day and night, but to other causes as well, and it may be experimentally shown that a variation of light intensity *often*, but not always, influences the rate of growth not only in organs which require a certain amount of light for their development, but also in those which can grow in complete darkness. This fact becomes very obvious if we compare the rate of growth in darkness with that taking place under feeble illumination. We may take a few examples first of all from organs capable of growth in continuous darkness. STAMEROFF (1897) determined the increase in growth by means of a micrometer in the following structures, which, under constant temperature, were exposed to the light of an electric lamp and darkened every 10–15 minutes alternately.

	Time of exposure.	Dark.	Light.	Dark.	Light.	Dark.	Light.	Dark.	Light.	Dark.
Mucor vegetative cells	10′	7	7	7	7	7	7	7	7	7
,, conidiophores	15′	10	9	9·5	8·75	9·25	8·5	9·25	8·25	—
Marchantia, roothairs	10′	6	4·5	6·25	4·5	6·25	4·5	6·25	4	—
Robinia, pollen-tubes	15′	—	6	6	6	6	6·5	6	6	6

The growth of pollen-tubes and of the ordinary hyphae of *Mucor* was by experiments of this kind shown to be unaffected by light, while the conidiophores of *Mucor* and the rhizoids of *Marchantia* often exhibited, as a result of illumination, a *very considerable* diminution in growth. Similar retardations due to light have been shown to occur in stems, leaves and roots of the higher plants by SACHS (1872), PRANTL (1873), STREHL (1874), and KNY (1902). KNY compared roots which had been exposed to *lights of varying intensity* with those which had been kept in the dark continuously for the same time, and found that after the lapse of several days the illuminated roots were markedly shorter than those which had been in darkness. Most of the authors mentioned above have compared—under constant temperature conditions—the increase in growth taking place during the night with that occurring by day. The influence of *brief* alternations of darkness and light in Phanerogams has been studied by REINKE (1876), who found that the hypocotyl of *Helianthus* exhibited in every quarter of an hour (in μ) the following increments :—

Darkness.	Light.	Darkness.	Light.	Darkness.	Light.
125	60	120	54	116	71

This diminution of growth in consequence of light, as already noted, may amount in individual cases to a complete cessation of growth, and a sufficiently great intensity of light may in the long run produce this result in every plant. Generally speaking, however, ordinary daylight, if it be acting *continuously*, produces merely a retardation and not an arrest of growth. Observations made on plants in the arctic regions, as well as the experiments which BONNIER (1895) has carried out in our own latitudes with artificial light, prove this conclusively.

It does not necessarily follow that the final shape and size of the plant should be influenced by the accelerating and retarding stimuli which we have hitherto dealt with; but as a matter of fact they are often actually so affected, and thus we may speak of a *formative* influence of light. At the same time we have to discriminate between the *intensity*, the *direction*, and the *quality* of the light which falls on the plant [especially the distribution of light on its upper surface].

The formative influence of continuous darkness has been longest known and is the most striking of the formative effects. Apart altogether from the alteration in colour which frequently takes place, plants which are grown in darkness exhibit special peculiarities in shape which are summed up under the term ' etiolation '.

The pure etiolating effect of darkness is seen naturally only when light is

I *II*

Fig. 88. *Dahlia variabilis.* *I*, grown in light; *II*, grown in darkness. From a photograph. Equally reduced.

withdrawn, other factors remaining constant. One of the indirect results of placing a green plant in darkness is to cause a cessation of carbon-dioxide assimilation and a constant stoppage of nutritive supply. In studying the various phenomena of etiolation, therefore, we must arrange that no such absence of nutrients takes place, and hence we employ plants for dark cultures which are abundantly provided with reserves (e. g. seeds, tubers, trees). If now we compare an etiolated shoot of *Dahlia variabilis* (Fig. 88, *II*) with one grown in light

(Fig. 88, *I*), we find that the internodes and petioles of the former have become greatly elongated, while the leaf-blades remain small and undeveloped. Microscopic investigation shows that the leaves remain in their embryonic condition, the tissues exhibiting little differentiation. Further, the last phase of growth in the stem is incomplete, for the mechanical elements are wanting, and hence the etiolated shoot is quite soft ; apart from this the individual cells are very much more elongated than in the normal shoot and their number is much greater. The majority of Dicotyledons with long internodes behave in the same way, but even in the case of the so-called 'rosette plants', such as *Sempervivum* (Wiesner, 1891 ; Brenner, 1900), etiolation results in the diminution in size of the leaves, the elongation of the condensed internodes, and in an opening out of the leaf rosette (Fig. 89, *III*). These phenomena are not universal, however ; organs which grow normally in the dark, naturally react differently from those which grow in light. Thus it would not be possible, for example, to cause an extension in subterranean bulbs with condensed internodes. Bulbous plants, e. g. bulbous species of *Oxalis*, exhibit totally different etiolation phenomena. In their case elongation of the stem does not take place, but the petioles of the leaves, on the other hand, elongate very considerably, while the leaf-blade remains small (Jost, 1895). The petioles of *Oxalis deppei*, for example, growing in the dark, but not full grown, were fifty-eight to seventy-eight cm. long, while the controls which were standing in a room in moderate sunlight, had petioles from eighteen to twenty-three cm. long.

Fig. 89. *Sempervivum assimile*. *I*, grown under normal conditions; *II*, grown in a moist atmosphere; *III*, grown in darkness. After Brenner (1900).

Many Monocotyledons, whose stems are outstripped by their leaves in rapidity of growth, behave in the same way as do species of *Oxalis*. Such plants, both in light and in darkness, form shoots of about the same length, but in the dark the leaves, owing to a continued capacity for growth in their basal meristems, exhibit a marked increase in length in the dark, but are generally smaller than when grown in light.

It has been customary to distinguish these two types of etiolation as the monocotyledonous and dicotyledonous respectively. In both of these groups of plants, however, there are numerous exceptional cases, in which etiolation does not take place or where the plants in question behave in the etiolated condition quite differently from their allies. Among plants which do not elongate their axis in the dark are certain climbers such as *Humulus* and *Dioscorea*, but their behaviour becomes intelligible when it is remembered that climbing plants form, to start with, very long internodes in light, with leaves which for a long time remain small in size. Further, plants are known in which the leaf-blades are not actually smaller in the dark than in the light, such as *Beta*, *Taraxacum* and *Tragopogon*. As already noted no elongation of the shoot takes place in bulbous species of *Oxalis*, while among Monocotyledons *Tradescantia* behaves quite like a Dicotyledon ; the leaves remain small and the internodes become elongated. Again among the grasses the Paniceae—e. g. *Zea mais*—have much elongated hypocotyls, and the leaves of hyacinths are smaller and shorter in the dark than in the light. Finally in the Cactaceae shoots grown in the dark remain shorter, often considerably so, than when grown in light (Vöchting, 1894 ; Goebel, 1895).

Etiolation is not limited to Monocotyledons and Dicotyledons ; it has been observed also in Gymnosperms, ferns, mosses, Algae, and Fungi. As to some of these cases we shall have something to say later on—at present a few examples only, taken from the Fungi, need be referred to (compare PFEFFER, Phys. II, 102). The influence of darkness is very marked on certain species of *Coprinus*, where a vigorous elongation of the stipe and a diminution of the pileus occurs. In extreme cases, in some species, the pileus is entirely suppressed (e. g. *C. stercorarius*), a result which can no longer be considered as a case of etiolation. An excessive elongation of the sporangiophore has been observed in many Mucorineae (*Pilobolus*), and the stalk of the perithecium of *Sphaeria velata* has been known in darkness to elongate to five times its usual length.

The instances of etiolated Fungi last cited are of especial interest when we attempt to answer the question as to the *causes* of etiolation. In these cases the accessory action of light (in carbon assimilation) is quite excluded from consideration. We can certainly prove that darkening the higher green plant does not induce etiolation by stopping carbon-dioxide assimilation. If we cultivate an autotrophic plant in light, but in an atmosphere without carbon-dioxide, we exclude all carbon-dioxide assimilation, yet no etiolation is to be observed.

The varied behaviour of the different organs of plants, as well as that of different species, shows also that in etiolation we have to deal with a *stimulatory* action of light, which under different conditions leads to the most diverse results. In the first place we have an alteration in the normal correlation of growth between organs ; the great development of the internodes hinders, in Dicotyledons, the usual surface-growth of the leaf-blade. Similarly we can induce the formation in darkness, in *Phaseolus* and *Mimosa*, of leaves of about normal size (JOST, 1895) if, by removing all lateral buds before their elongation, the main shoot is saved from competition with them. PALLADIN (1890) has obtained the same result by retarding growth in the internodes by appropriate means. It is not, however, known in detail how illumination or darkening operates on the growth of cells, and the numerous experiments which have been made lay stress for the most part on *one* possible factor only, such as turgor, elasticity of the cell-wall, &c., thus implying that the problem is simpler than it really is. It must be remembered amongst other things that etiolation is not a simple consequent of light activity, but is rather a complex result of several secondary influences, especially the retardation of transpiration (compare p. 319).

We know much more about the biological significance of etiolation than we do about its *causes* (GODLEWSKI, 1889 ; DARWIN, 1896). Looking upon superelongation of certain organs as the most usual characteristic of etiolation we may regard it as an adaptation on the part of the plant to escape from darkness. From this point of view it is immaterial whether the internodes or the petioles elongate ; the chief point is that the special organs which require illumination should be lifted out of the darkened area. The leaves need not enlarge if they are not to exercise their functions. Further investigation is required to show how far the superelongation of the fruit-bearing parts of Fungi or mosses is of service. In the case of *experiments* carried out in the dark etiolation is certainly of no service ; in their natural *habitat*, however, originally underground shoots or creeping parts, which are covered with earth or leaves, again reach daylight in virtue of the effect of etiolation coupled with geotropism (Lecture XXXIV). Owing to the combined action of etiolation and heliotropism (Lecture XXXVI), the plant is able to put itself in a position to reach the most suitable light intensity. Etiolation is not induced by absolute darkness only ; diminution of light has a similar effect, and generally speaking each varying intensity of light to which a plant is subjected, impresses itself on its structure. As light increases the leaf grows in size up to a certain maximum, while higher illumination causes a diminution in size. The stem behaves con-

versely, and BERTHOLD (1882) has demonstrated superelongation in Algae placed in somewhat too bright light. Diminution, however, may clearly be seen to follow further increase in light intensity, finally resulting in a cessation of growth. It is not difficult to prove an increase in leaf-surface as a concomitant of an increase in illumination if we compare etiolated with normal leaves. STAHL (1883) has, however, calculated that in moderate light in shady situations leaves become larger than in direct sunlight, and has shown that beech-leaves, exposed to sunlight are only half, and leaves of the elder only a quarter, the size of those grown in the shade. Thickness of the leaf stands in close relation to area ; the thickness increases with the reduction of surface and vice versa. It is known also that the anatomical structure of the leaf is greatly affected by light. Elongated palisade tissue is the characteristic feature of leaves exposed to sunlight, spongy mesophyll of leaves which are shaded. While many plants demonstrate themselves to be light or shade plants by their leaf anatomy there are other important adaptations which are worthy of note (Fig. 90).

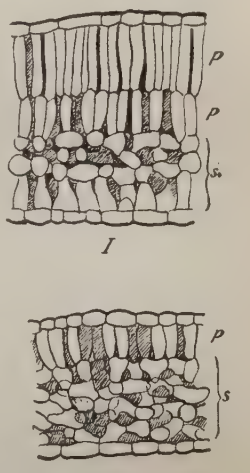


Fig. 90. Transverse sections through the leaves of the copper beech. *I*, illuminated ; *II*, shaded. After NORDHAUSEN (1903, Pl. 4).

Experimentally it may be shown that each bud can be made to unfold, and in doing so, if the light be sufficient, it becomes a normal shoot, if insufficient an etiolated one. In nature weakly illuminated buds do not produce etiolated shoots, they simply do not develop at all. Evolution of the bud takes place only if the light be sufficiently intense, and the degree of intensity varies greatly for different plants. WIESNER (1893–1900, summary, 1902) has provided us with accurate measurements on the subject which lead us to numerous important results. A few of these only can be quoted on the present occasion. [Compare WIESNER, 1904 and 1905 ; CIESLAR, 1904 ; HESSELMAN, 1904.] WIESNER used the BUNSEN-ROSCOE method for measuring light intensity, a method well adapted for estimating the highly refrangible rays only, i. e. those which are of most importance to the plant (p. 311). WIESNER has determined the degree of light intensity under which a number of plants will live in different surroundings. First he determined, by the BUNSEN-ROSCOE method, the 'absolute photic ration', and then deduced therefrom the 'relative photic ration' falling on the plant. If the plant is able to live, on the other hand, in full sunlight, and also in a light intensity reduced to one-tenth of the maximum, WIESNER says the relative photic ration lies between one and one-tenth.

WIESNER gives the following data for Vienna :—

	Relative photic ration.	Minimum of absolute photic ration calculated by the BUNSEN-ROSCOE method.
Buxus sempervirens	1 to $\frac{1}{100}$	·012
Beech (enclosed)	1 to $\frac{1}{80}$	·015
„ (open ground)	1 to $\frac{1}{60}$	·021
Quercus pedunculata	1 to $\frac{1}{20}$	·050
Betula verrucosa	1 to $\frac{1}{9}$	·144
Larix decidua	1 to $\frac{1}{5}$	·20

For one and the same species the higher the latitude or the altitude the more the light that is required. Its absolute and relative photic ration increases with the diminution of temperature. Thus the minimum of the relative photic ration in the case of *Acer platanoides* alters from one-fifty-fifth near

Vienna to one twenty-eighth at Drontheim, and to one-fifth at Tromsö; the minimum of the absolute photic ration in *Betula nana* is 0·338 at Christiania, 0·386 at Tromsö, and 0·750 at Spitzbergen.

Wiesner draws the following conclusion from his observations:—'*Just as the plant requires a certain amount of heat for the proper performance of its functions, so also it needs a certain definite amount of light.*' It has not, however, been proved that the plant requires a certain *amount of heat*, we only know that it needs a certain *degree of temperature*. Unless this be provided it is not only impossible for it to *thrive* but all growth is impossible. Branches of the above-mentioned plants can also grow without any light, and so light cannot be considered as an absolutely necessary condition of life in the same way as temperature (p. 301).

The formative influences of light hitherto discussed refer to disturbances in the normal correlations of organs, and express themselves in increase on one side and decrease on the other; when total exclusion of light is effected and the plant becomes etiolated these phenomena are most clearly marked. When, however, we speak of an etiolated plant yet another modification of the normal plant occurs to us, viz. alteration in *colour*. Etiolated plants have white stems and yellow leaves, since chlorophyll is not usually formed in darkness. We may with good reason, however, distinguish between this colour change and real etiolation, by which we understand only 'excessive elongation' and 'reduction,' since etiolation may take place in cases where chlorophyll is present. Later on we shall become acquainted with factors, other than darkness, which induce excessive elongation without injuring the chlorophyll. Moreover, there are quite a number of plants in which the formation of chlorophyll is independent of light and in which all the same a marked etiolation takes place in darkness (Schimper, 1885). This appears to be the case generally with Algae and mosses, while Pteridophyta behave variously. The Equisetaceae, like Phanerogams, form no chlorophyll in the dark, whilst the Filicinae do. The Gymnosperms are especially interesting; while the adult plant can form no chlorophyll in the dark, the seedlings of Coniferae and of *Ephedra* have that power (Sachs, 1862 and 1864; Burgerstein, 1900). In cases where the formation of a yellow-colouring matter only takes place in chloroplasts in the dark, it is often quite sufficient to expose leaves which are not too old for a short time to sunlight in order to obtain the green coloration. If we again place the plant in darkness, after the light has acted for a time on the etiolated leaf, but before the light has produced any visible effect, the greening takes place in the dark as an after-effect. A very *low* light intensity is sufficient for the purpose, greater intensities indeed tend to destroy the chlorophyll.

All vegetable colouring matters do not behave like chlorophyll. Sachs (1863) showed that many plants formed normally coloured flowers in darkness (e. g. tulip, crocus, cucumber, &c.); in other cases, however, these floral colours are formed only in light (Askenasy, 1876). Similarly the formation of the red-colouring matter, often found dissolved in cell-sap, depends entirely on the presence of light (Overton). In all these examples we are dealing with effects of light which may influence very greatly the general appearance of the plant, but which are perhaps quite simple phenomena, to be explained by a sufficiently thorough knowledge of the chemical composition of the colouring matter in question. We have still, however, other formative influences of light to consider which present more difficult problems for solution.

There are many plants of varied relationship which exhibit a different form when young from that exhibited by them when more fully developed. In many of these the juvenile form is produced by being exposed to light of less intensity than in older stages. If the intensity of light remains low, the later form is suppressed; if the intensity of light diminishes after the adult

form has appeared, the plant reverts to the juvenile condition once more. The following may be cited as examples of this phenomenon. The alga *Batrachospermum* has a juvenile form named *Chantransia* (GOEBEL, 1889); protonemata of mosses (KLEBS, 1893), and the elongated and circular leaves of *Campanula rotundifolia* are examples of the same phenomenon (GOEBEL, 1896, Fig. 91).

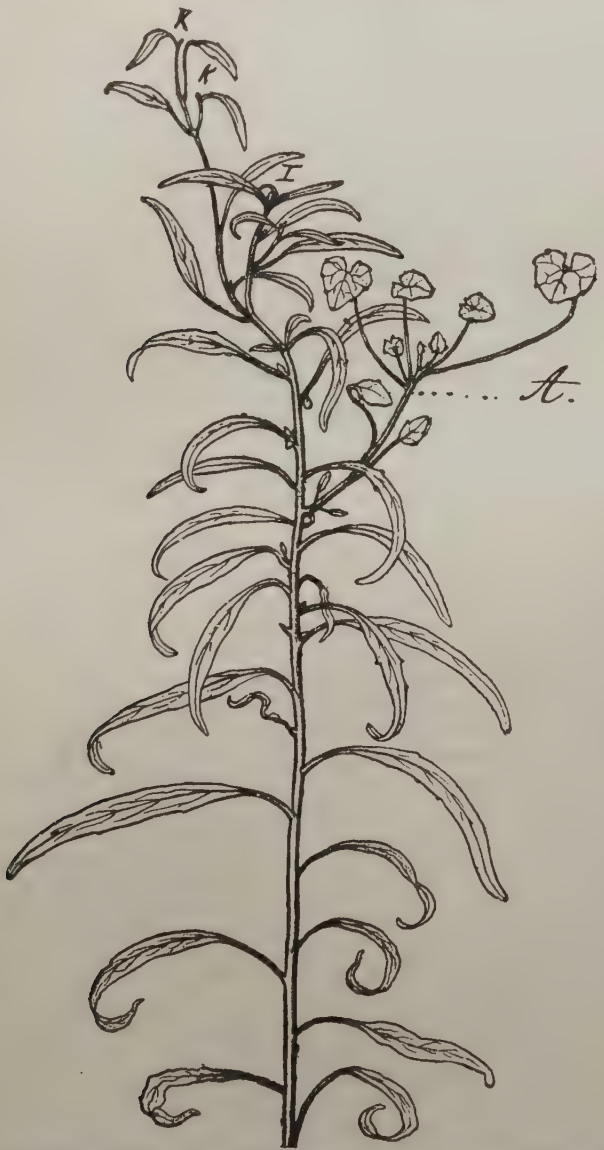


Fig. 91. *Campanula rotundifolia*. When feebly illuminated the flower-buds *k* are arrested; a lateral shoot *A* develops circular leaves. From GOEBEL'S Organographie.

Certain Cactaceae (*Opuntia, Phyllocactus*) may be added to this list, since their shoots *flatten* only in light and revert to the original radial stem-like symmetry in the dark (GOEBEL, 1895; VÖCHTING, 1894).

Since the several organs of plants often bear the most varied relations to

light intensity, the origin or development of an organ may be stopped by insufficient illumination, or may be guided in other directions, so as to present in the long run a totally different appearance. The Fungi form good examples of this. The formation of sporangia does not take place in the dark in *Pilobolus microsporus*, while the sterile sporangiophore grows far beyond the normal length, and an analogous phenomenon in *Coprinus* was referred to above (GRÄNTZ, 1898). Let us glance next at the behaviour of the roots in the higher plants. In many cases these develop only in darkness, and hence aerial roots may appear in etiolated plants, roots which are entirely wanting in plants when exposed to light, even though they be cultivated in a damp atmosphere. On the other hand, light stimulates the formation of buds and thus (WIESNER, 1895, 685) buds are produced on the upper side of the downwardly-bent branches of the willow, which are mostly illuminated from above, and on the underside of the upright branches of the poplar, which are more illuminated from below. Very remarkable effects of light have been observed in the case of subterranean shoots. The offsets of *Circaea* form scale-leaves in the dark but foliage-leaves in the light (GOEBEL, 1880); the stola of *Adoxa* may be made to grow greatly in length if exposed to light, while darkening them at once induces a stoppage of longitudinal growth and a formation of tubers (STAHL, 1884). Further, the development of potato tubers is associated with darkness, and it is possible by appropriate means to induce the formation of tubers even on the top of the leafy shoot (VÖCHTING, 1887). Perhaps the most interesting case is that described by BERTHOLD (1882); he was able by weak illumination to induce the formation of roothairs from the apex of the shoots of many Algae (*Callithamnion, Bryopsis*). In *Bryopsis* the pinnae also were transformed into roots in the dark (NOLL, 1888 and 1900; WINKLER, 1900 a).

We shall have another opportunity of studying the effect of the intensity of light on flower formation, at present we have still to glance at the influence of the *direction* of light on the structure of organs. Unilaterally incident light, i. e. illumination of different parts of the plant with light of unequal intensity, often produces formative results well worthy of notice. Very frequently in plants which have developed polarity, light-direction determines which end is to be base and which apex, which root and which shoot. In *Equisetum*, according to STAHL (1885), the first plane of division in the germinating spore is formed at right angles to the path of the incident ray, thus separating a shaded root-cell from the illuminated prothallus-cell. WINKLER (1900 b) has observed a similar phenomenon to occur in the egg-cells of *Cystoseira barbata*. He was also able to determine the time required to develop this result; thus unilateral illumination for three hours had no effect, but four hours' exposure was sufficient to produce a result after the plant had been many hours in darkness. Germination and a differentiation of root and shoot also occurred, though delayed, without any such unilateral illumination. Many lower plants behave in the same way, while in others, and especially in the higher plants, polarity is undoubtedly independent of external conditions.

The symmetry of the plant body is also dependent on the direction of light, being radial when the light falls equally on all sides and dorsiventral when the light is unilateral. Thus in *Antithamnion cruciatum* the branches are approximately decussate in diffuse light, but if illuminated on one side only they all arrange themselves in one plane perpendicular to the path of the incident ray. Other structures are *always* dorsiventral and the light determines only which side shall be the upper, which the lower. Examples of this are seen in the shoots of *Lepismium radicans* and *Hedera helix*, where roots are formed on the shaded side only; the rhizome of *Caulerpa* forms roots on the shaded and shoots on the illuminated side, in fern prothalli roothairs and sexual organs are formed on the shaded side, and in the thallus of *Marchantia* rhizoids are formed underneath and assimilatory parenchyma above. The direction of the light determines

dorsiventrality in higher plants also, e. g. in *Thuja* (FRANK, 1873), and *Begonia* (ROSENVINGE, 1889). In most cases it is possible by altering the direction of the light-incidence to produce corresponding changes in the dorsiventrality; thus fern prothalli may be made to form sexual organs on the upper side by illuminating the lower, but the dorsiventrality in *Marchantia* is so fixed by heredity that new growths are no longer affected by external factors, but follow in their development the plan of structure already laid down by the fully-developed regions.

We must content ourselves with these examples and in conclusion inquire as to the significance of the *quality* of light, the colour or wave-lengths of the rays. We arrive at this most important fact that in growth and formative processes the more refrangible rays are those which are operative. In many cases it has been shown that the less refrangible rays which do most of the work in carbon-dioxide assimilation act morphogenetically like darkness. We see, therefore, how little the cessation of carbon-dioxide assimilation has to do with etiolation, since green plants become etiolated in *red* light in spite of assimilation taking place at the same time. The formation of chlorophyll goes on equally well in light of all wave-lengths (REINKE, 1893). [Other pigments behave differently; in *Oscillaria*, according to GAIDUKOW'S (1903) experiments—which wait confirmation however—a so-called complimentary adaptation of colour takes place, that is to say, the cells become red in blue light and blue in red light.]

For long it was believed that ultra-violet light had quite a special effect on the external formation of the plant. SACHS'S (1887) statements on the point have not, however, been confirmed (compare p. 364). The influence of Röntgen and of other recently-discovered rays, has been studied, but without leading to results of any physiological value. [Compare KÖRNICKE, 1905.]

Bibliography to Lecture XXIV.

ASKENASY. 1876. Bot. Ztg. 34, 1.
BERTHOLD. 1882. Jahrb. f. wiss. Bot. 13, 569.
BONNIER. 1895. Revue gén. de bot. 7, 241.
BORODIN. 1868. Bull. Acad. Pétersbourg, 13, 432.
BREFELD. 1877. Bot. Untersuchungen üb. Schimmelpilze, 3, 93.
BRENNER. 1900. Flora, 87, 23.
BROWN and ESCOMBE. 1895. Proc. Roy. Soc. 62, 160.
BURGERSTEIN. 1900. Ber. d. bot. Gesell. 18, 168.
[CIESLAR. 1904. Centrbl. f. d. Ges. Forstwesen. 1.]
DARWIN, F. 1896. Journal Roy. Hort. Soc. 19 (Bot. Ztg. 1896).
FRANK. 1873. Jahrb. f. wiss. Bot. 9, 147.
[GAIDUKOW. 1903. Scripta Horti Petropol. 22, 7.]
GODLEWSKI. 1889. Biolog. Centrbl. 9, 481.
GOEBEL. 1880. Bot. Ztg. 38, 794.
GOEBEL. 1889. Flora, 72, 1.
GOEBEL. 1895. Ibid. 80, 96.
GOEBEL. 1896. Ibid. 82, 1.
GRÄNTZ. 1898. Einfl. d. Lichtes auf die Entw. einiger Pilze. Diss. Leipzig.
HEALD. 1898. Bot. Gaz. 26, 25.
HEINRICHER. 1899. Ber. d. bot. Gesell. 17, 308.
[HESSELMAN. 1904. Zur Kenntniss des Pflanzenlebens schwedischer Laubwiesen. Jena.]
HILBRIG. 1900. Einfl. supramax. Temp. auf d. Wachstum. Diss. Leipzig.
JOST. 1895. Jahrb. f. wiss. Bot. 27, 403.
KLEBS. 1893. Biol. Centrbl. 13, 641.
KNY. 1902. Jahrb. f. wiss. Bot. 38, 421.
KÖPPEN. 1870. Wärme u. Pflanzenwachstum. Diss. Moskow.
[KÖRNICKE. 1905. Ber. d. bot. Gesell. 23, 324 and 404.]
LEITGEB. 1876. Sitzungsber. Wiener Akad. 74.
LEITGEB. 1886. Mitt. a. d. bot. Instit. Graz, 1, 123.
[MEZ. 1905. Flora, 94, 89.]
MÖBIUS. 1895. Biol. Centrbl. 15, 1.

Molisch. 1897. Unters. üb. das Erfrieren d. Pflanzen. Jena.
Müller-Thurgau. 1886. Landw. Jahrb. 15, 453.
Noll. 1888. Arb. bot. Inst. Würzburg, 3, 466.
Noll. 1900. Ber. d. bot. Gesell. 18, 444.
Nordhausen. 1903. Ber. d. bot. Gesell. 21, 30.
Overton. 1899. Jahrb. f. wiss. Bot. 33, 171.
Palladin. 1890. Ber. d. bot. Gesell. 8, 364.
Popovici. 1900. Bot. Centrbl. 81, 33.
Prantl. 1873. Arb. bot. Inst. Würzburg, 1, 371.
Raciborski. 1900. Bull. Inst. de Buitenzorg, No. 6.
Reinke. 1876. Bot. Ztg. 34, 143.
Reinke. 1893. Sitzungsber. Berliner Akad. 527.
Rosenvinge. 1889. Revue gén. de bot. 1, 153.
Sachs. 1862 and 1864. Flora, 45, 186; 47, 505.
Sachs. 1863. Bot. Ztg., Beilage.
Sachs. 1864. Flora, 47, 8.
Sachs. 1872. Arb. bot. Instit. Würzburg, 1, 99.
Sachs. 1887. Ibid. 3, 371.
Schimper. 1885. Jahrb. f. wiss. Bot. 16, 1.
Stahl. 1883. Jen. Zeitschr. f. Naturwiss. 16.
Stahl. 1884. Ber. d. bot. Gesell. 2, 389.
Stahl. 1885. Ibid. 3, 334.
Stameroff. 1897. Flora, 83, 135.
Strehl. 1874. Längenwachstum d. Wurzel u. des hypocotylen Gliedes (Diss. Leipzig).
Thiselton-Dyer. 1899. Proc. Roy. Soc. 65, 362.
Vöchting. 1878. Organbildung im Pflanzenreich, I. Bonn.
Vöchting. 1887. Bibliotheca botanica, Heft 4.
Vöchting. 1894. Jahrb. f. wiss. Bot. 26, 438.
Vöchting. 1902. Bot. Ztg. 60, 87.
Wiesner. 1873. Sitzungsber. Wien. Akad. Math.-nat. Kl. 67, 1, 9.
Wiesner. 1891. Ber. d. bot. Gesell. 9, 46.
Wiesner. 1894. Sitzungsber. Wien. Akad., Math.-nat. Kl. 103, 401.
Wiesner. 1893, 1895, 1900. Photometr. Untersuch. auf pflanzenphys. Gebiete. Sitzungsber. Wien Akad. Math.-nat. Kl. 102 (1893), 104 (1895), 109 (1900).
Wiesner. 1902. Biologie d. Pflanzen. Vienna.
[Wiesner. 1904. Sitzungsber. Wiener Akad. 113, T. 469.]
[Wiesner. 1905. Ibid. 114, T. 77.]
Winkler. 1900, a. Jahrb. f. wiss. Bot. 35, 449.
Winkler. 1900, b. Ber. d. bot. Gesell. 18, 297.

LECTURE XXV

EXTERNAL CAUSES OF GROWTH AND FORMATION. II

In addition to heat and light, *gravity* must be considered as a factor frequently influencing the growth and shape of plants. To begin with it may be well to illustrate by means of a few examples that the *weight* of the entire plant or of its parts is not only not injurious, but is even helpful to vitality. Great weight, absolute or relative, is naturally, for purely mechanical reasons, a disadvantage in the distribution of seeds, and hence we find developed in many cases floats, wings, or other adaptations of the most varied character. In aquatic plants also we find the vegetative organs provided with aids to flotation, for the water has to support the weight of the organs which terrestrial plants themselves support. Land plants possess, indeed, special mechanical tissues which are absent from aquatics. Twining and climbing plants exhibit special adaptations (Lectures XXXV and XXXVIII), e. g. holdfasts of various kinds which render the development of a special strengthening skeleton more or less unnecessary.

All these adaptations are special peculiarities of the plant as such, which are in no respect due to the direct influence of gravity on the individual.

More important for us are the accessible effects of gravity as shown by experiment. Further, in these effects also it is often that of the weight of an entire organ that is concerned, a tension or pressure, and in this case gravity is of course replaceable by other forces. On the other hand, gravity exerts a specific influence which, even when the weight of the entire organ is eliminated by being supported by a prop or by immersion in water, makes itself felt both in cells and cell parts. We will begin our studies with a consideration of such specific effects of gravity; the effects of pressure and tension we may postpone till later.

The specific effect of gravity exhibits itself especially in the *direction* which plant organs assume in space, and to which they return if they be displaced. These movements are produced by changes in shape, which we will consider in another connexion (Lectures XXXIV and XXXV). It may be remarked, however, that both in these movements and also in the effects on growth and formation to be described here, gravity may be replaced by centrifugal force, from which we may conclude that it is the 'mass-acceleration' which directly or indirectly affects the plant. The fact that gravity may be replaced by centrifugal force suggests the question as to the effect of the intensity of the acceleration. ELFVING'S (1880) and SCHWARZ'S (1880) experiments have shown that growth is not affected by increasing or reducing the acceleration, but disturbances in the arrangement of the cell-contents are induced by great centrifugal force (MOTTIER, 1899), and disturbances in growth are also to be expected.

In nature the question of the intensity of mass-acceleration is of little consequence, because the differences in the value of gravity on the earth's surface are too small to be worth notice. The *direction* in which gravity acts on the plant is of far more importance. Very frequently the *symmetry* of the plant part depends on how it lies with regard to gravity, being *radial* when its long axis is parallel with the direction of gravity, and *dorsiventral* when it is not so. Dorsiventrality exhibits itself in the distribution and formation of the lateral branches; on the dorsiventral stem the branches and leaves are usually produced either on the upper side only, or if they can develop all round, those on the under side are distinguished by size from those on the upper (anisophylly). Roots also appear to be developed only on the underside of dorsiventral organs. Dorsiventrality may be recognized either at the growing point by the position of the *primordia* of organs, or later on in the course of development of such organs. The latter phenomenon is very common and is especially well seen in the cuttings of the willow for example (VÖCHTING, 1878). If such a cutting be suspended in its normal position in a moist chamber it forms at its apex radially arranged lateral shoots, and similarly disposed roots at the base, but the median region develops no lateral branches. If the cutting be placed *horizontally* branching takes place at apex and base as before, but a number of lateral shoots also appears on the *upper surface* from the apex backwards, and, further, numerous roots arise from the *under surface*; in other words the branch has become dorsiventral. Finally, if the branch be suspended upside down, its apex downwards and base uppermost, the largest shoots and roots appear as in the first experiment, at the apex and base respectively, but both types of organ develop progressively towards the opposite pole as in the normal orientation. In this case a very obvious effect of gravity is disclosed, but it has no power to alter the pre-existing polarity. This is very well shown in the case of certain cultivated arboreal forms, e. g. the so-called 'weeping trees'. The pendent branches of these plants, in spite of their inverted position, continued to form lateral branches at their apices (VÖCHTING, 1878).

As yet we know of no case where gravity has induced polarity in the growing point or in the ovum, as, for example, light is capable of doing in *Equisetum*; the possibility of such an effect, however, cannot be denied. The examples

quoted might be largely added to, still, on the whole, the *morphogenetic* influence of gravity, much over-rated in HOFMEISTER'S time, is very limited. Gravity has nothing like the effect on plant shape that light has.

Growth in length, on the contrary, is in general markedly influenced by gravity. Thus it has been clearly established that *Chara* and *Phycomyces* (ELFVING, 1880; RICHTER, 1894) grow more slowly when inverted than when in the normal position, and other plants behave in a similar manner. [HERING (1904) has shown that this retardation of growth in consequence of inverted orientation is a phenomenon of widespread occurrence.] When shoots and roots are placed at an angle to the direction of gravity their upper and under sides grow at different rates, with the result that these members bend in a way which we shall have to study later (Lecture XXXIV).

Growth in thickness of trees is also not uniformly affected by gravity. In all sloping branches the upper side grows in thickness at a different rate from the under. In Coniferae (and in *Aesculus* also) the under side grows more in thickness than the upper side, while the converse takes place, at least at first, in Dicotyledons (WIESNER, 1895–6). Extensive investigations undertaken by HARTIG (1901) on the Coniferae show that the increased growth of the under side may occur in the main stem also when it is placed horizontally even when appropriately supported so that the action of mere weight on the under side is neutralized; the effect can be attributed under these conditions only to the direct influence of gravity. Further, the under side exhibits not merely an increase in secondary thickening, but also a special anatomical structure in the wood formed ('redwood'), attributed also to the direct action of gravity by HARTIG.

HARTIG further attempted to show that the increased growth and formation of 'redwood' arose from *pressure* longitudinally exerted on the cambium, which must arise in general in horizontally-placed branches owing to the influence of mere weight. Similarly, feebler increase in thickness and characteristic anatomical structure of the wood on the upper side may be accounted for by the *tension* in the longitudinal direction to which the cambium is exposed in that region. HARTIG has given us no direct proof of this conjecture, but no one can deny that this explanation is an extremely probable one, for it has often been observed that tension and pressure have a distinct influence on growth.

Pressure exerted on growing cells must retard their growth and may finally completely stop it. Cells so prevented from growing exert on their part a pressure on the surrounding tissues, often leading to quite obvious mechanical results. As PFEFFER (1893) has shown, this outward pressure is brought about in this way—the cell-wall becomes relaxed through surface-growth, and the whole osmotic pressure is thus directed against the external opposing layer. In individual cases an increase in the osmotic pressure may also be observed under such conditions. Frequently the plant is able in this way to overcome external resistance, as, for example, in the splitting of rocks by roots.

Tension of necessity acts on cells in the reverse manner to pressure. An increase in growth in the direction of the tension is to be expected, and that such takes place is easily proved if a stem be stretched by a weight. The pull has, however, at the commencement quite a different effect; it acts as a stimulus and induces a retardation in growth, followed later on by the acceleration mentioned (HEGLER, 1893).

The new cell-walls formed in cell division show many relations to tensions and pressures exerted on them; the planes of division appear parallel to the line of action of the pressure and transversely to that of the tension, provided there be no other circumstances to prevent that result (KNY, 1901). A further stimulative effect of tension is seen in the mode of formation of the internal tissues. In ripening fruits under natural conditions, and in experiments on other organs, the amount and also the thickness of the mechanical elements increases in pro-

portion to increase in weight. [KELLER (1904) has recently shown that in the peduncles of ripening fruits there is an increase in the tendency to rupture, but he (as well as WIEDERSHEIM and BALL) has shown that this is not due to increased weight.] Hence we obtain morphogenetic responses as the result of the action of mechanical factors, responses which are frequently observed in other cases. Thus, by bending of the main root the development of lateral roots from the concave side may be prevented, they then arise only on the convex or stretched side (Fig. 92, NOLL, 1900). Very often we may observe results of pressure manifesting themselves with different intensity in adjacent parts of the same organ. Such 'contact sensitivity' is illustrated by roothairs, which exhibit a diminution of growth when in contact with the soil particles, accommodating themselves in the most complete way to the inequalities of surface. Contact responses of a special kind are exhibited by the tendrils of species of *Ampelopsis* which form holdfasts by pressing their apices in close contact with some solid body (LENGERKEN, 1885). Lastly *Mucor stolonifer* produces stola whose apices, when they come in contact with the substratum, attach themselves to it by means of rhizoids, the plant thereafter proceeding to form sporangiophores (WORTMANN, 1881).

The examples cited must suffice to illustrate the influence of pressure and tension on growth, and we must now turn to the consideration of certain other external influences on growth which act sometimes chemically, sometimes mechanically. Since all growth is dependent on the supply of nutritive substances, it follows that the essential nutrients we have already referred to may be considered as conditions of growth, and each of these must be present in a certain minimum quantity before growth takes place. An excess of the other nutrients does not compensate the plant for the deficiency in one. Under such starvation conditions growth results for the most part in a diminution in the size of the whole plant. HEINRICHER (1896) has described plants of *Sinapis nigra*, which when sown closely on bad soil reached a height of only 18 mm., but which formed both flower and fruit. Similar dwarf plants were obtained by LÜPKE (1888) in cultures without potassium; a similar case is shown in Fig. 93. The reduced size, which many plants exhibit in the absence of nutritive materials, may be considered as a phenomenon of adaptation, since the organism is able in this way to complete its life-cycle, while, by employing the nutrients available for the formation of organs of normal size, only a single leaf perhaps could be formed, and the development would then come to an end. The dwarfing of all the organs, i. e. *harmonic* dwarfing, as we may quite correctly term it, is, however, not the only response given by the plant to absence of nutrients; in some cases inharmonic growth also results. We find, for example, that when *nitrogen is absent* roots, roothairs, and internodes undergo *excessive elongation* (NOLL, 1901; BENECKE, 1903). We may term this 'etiolation', and biologically it is obviously closely related to etiolation due to absence of light. As the individual nutrients

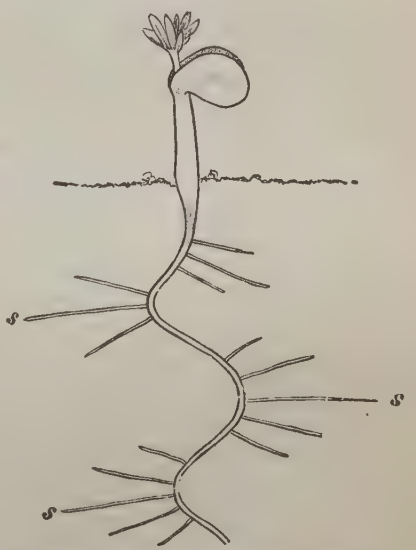


Fig. 92. Young lupin with a curved principal root. The lateral roots are developed exclusively on the convex sides. After NOLL. From the Bonn Textbook.

increase in amount we at length reach certain quantities of each which produce an *optimum* effect, and when these amounts are exceeded injurious results follow, due either to osmotic or to chemical influences, and resulting in death if a certain *maximum* be exceeded.

Amongst the substances which are essential to most plants oxygen is of special importance. It is not a 'food-stuff' in the ordinary sense of the term, since it is associated, not with constructive, but with destructive metabolism, e. g. with respiration. Growth is affected in a most marked manner by the degree of concentration of oxygen present. If care be taken that the air-pressure as a whole remains unaltered, *decrease* in oxygen pressure induces an acceleration of growth, so that the normal amount of oxygen in the air may be considered as supra-optimal so far as growth is concerned. In many cases, however, by *increasing* the partial pressure of oxygen an increase in the rate of growth may be observed, so that there would appear to be two optima as regards concentration of oxygen. Possibly this may be an indirect result of experimental conditions. In all cases, however, we are able to increase or decrease the amount of oxygen present in the air to such a degree as to retard growth and finally bring about death (maximum and minimum oxygen pressures). After what we have already learned as to the oxygen-requirements of different plants, the specific differences in minima and maxima of that gas are easily understood. A concentration which is subminimal to an ordinary aerophilous plant may be supramaximal to aerophobous ones such as anaerobes [compare PERODKO (1904)]. As transitional forms between these two extremes, the sulphur Bacteria are of special interest, because they have a very low oxygen-optimum, although oxygen is absolutely essential to them. While typical anaerobes require as a condition of *growth* a very low partial pressure of oxygen, genuine aerobes as a rule do not exhibit growth when the conditions are such as to induce intra-molecular respiration (WIELER, 1883 and 1901), or at most they show only a very insignificant increase in length (NABOKICH, 1901–2).

Fig. 93. Seedling rose grown in the Strassburg Botanic Garden, which after forming a few leaves proceeded to form flowers in the first year of growth (nat. size).

As already noted, many of the substances essential to plant life act injuriously when certain definite concentrations are reached, and if the injury be referable to the chemical action of the substance we may term it a 'poison'. Many metabolic products found in plants are in this sense poisonous both to the plants which produce them and to others as well. [NIKITINSKY (1904) has also discovered metabolic substances in Mould-fungi which accelerate growth in these plants.] Plants are in general capable of resisting the action of their own metabolic products, but only if they occur in limited amount. Retardation of development results in the long run from the presence in excess of alcohol or acids produced during fermentation, and so also in the higher plants the products appearing in them may act injuriously if they be not used up, e. g. carbon-dioxide, or be not made insoluble and hence innocuous, as, e. g., when oxalic acid unites with calcium. There are also substances, however, which never occur in plants and with which they do not come into contact in nature, which are virulent poisons, retarding growth even in very dilute solutions. It is unnecessary to enumerate these poisons here ; we need only note that many substances

are equally poisonous to plants and animals, while others affect even closely-related organisms in entirely different ways. This is explained in part by the fact that protoplasm is not identical in all organisms, but more especially that the relative *rate* of *entry* of the poison into the protoplasm is most varied. Thus PULST (1902) has shown that sulphate of copper, which is generally a virulent poison, like most of the salts of the heavy metals, has no effect on *Penicillium*, only because it is not absorbed by this fungus. It is quite inexplicable, however, why sugar and peptone, which are valuable nutrients to the majority of plants and not in themselves in any sense injurious, should be extremely poisonous to nitro-Bacteria (p. 229).

It is of special interest to recall the fact already mentioned that many poisons have not only no injurious effect in dilute solution, but are actually of service to the organism by stimulating its respiratory and metabolic activity.

We may also recognize that many substances act as chemical 'stimuli' which are not poisonous ; even nutrients may act as such, though that is not their chief function ; at all events this aspect of their activity is not to be associated with their nutritive value. Examples of these phenomena are seen in cases where growth is initiated by the presence of substances whose nature is in some cases known, in some cases unknown, and more especially in the germination of spores and pollen-grains. For example, the pollen-grains of certain species of *Mussaenda* germinate in distilled water, according to BURCK (1900), but only when a small portion of the stigma is added to the water. Apparently the stigma contains levulose, for of all the materials experimented with, and especially sugars, this was the only one which acted in this way even when merely very minute traces were present. It is not easy to understand why dextrose should not act in the same way if it be merely the addition of some material needed for growth that was wanted. If, however, levulose be considered merely as a stimulant to growth, then the extreme specialization becomes intelligible. Further, closely-allied forms show great differences in this respect ; the pollen of *Pavetta javanica* germinates only in an extract of the stigmas of that plant or of *Pavetta fulgens*, but not in that of other species. Finally, it may be noted that according to DE BARY (1884) spores of *Completoria*, *Protomyces*, and *Synchytrium* germinate as a rule only on their host-plants, and that *Orobanche* and *Lathraea* develop their haustoria only in the neighbourhood of the roots of their hosts. It cannot be doubted that in these cases also some growth stimulant of a definite chemical nature is given off from the host-plant, but such substances have not as yet been isolated.

That chemical stimuli are also able to act in a *formative* manner need not be further exemplified, since we have already seen (p. 249) how *Basidiobolus* behaves under such conditions. Later on (galls, p. 320) we shall have an opportunity of studying some morphogenetic results of chemical stimuli.

We may conclude this account of the effect of materials on growth by considering *water*, which in addition to its *chemical* effect has also undoubtedly an important *physical* influence on imbibition and turgescence, and hence on the elasticity and pressure phenomena of the plant. When water is withdrawn, as a rule all vital activities, including growth, come to an end, but certain plants retain capacity for life even in the desiccated condition. Many mosses, lichens, and even species of *Selaginella* are able to endure air-drying without suffering permanent injury, and to start growing again as soon as water is once more supplied. The majority of plants, however, die when once dried in the vegetative state. The capacity for withstanding desiccation is very general in the resting stages of plants, e. g. 'spores' and seeds ; and in many of the lower plants the actual *formation* of such bodies is dependent on the withdrawal of water. These resting stages can frequently withstand much higher degrees of desiccation than can be obtained by simple air-drying ; many seeds, for example, can withstand

drying induced by a temperature of 100° to 110° C. without being killed, while many mosses are killed by drying in a desiccator. As in general, so also in plants which live under special conditions of life, special peculiarities are developed, whilst plants whose seeds under normal conditions are never desiccated, frequently do not possess the *power* of withstanding drought.

Long before air-dryness is reached turgidity is destroyed, making itself evident by the wilting of the plant members. The capacity to endure wilting is also very varied among plants. Certain succulents can endure a loss of as much as 90 per cent. of the water they contain without suffering permanent ill effects, other plants again can only stand the loss of half the water they normally hold. When turgor is destroyed, growth over all comes to an end. The loss of water may be due either to transpiration under conditions when the supply of water is insufficient, or to osmotic action due to the presence of salt solutions which themselves produce no *chemical* effect. The results are not identical in the two cases, and this is quite intelligible for the reason that when water is withdrawn osmotically, as has been already pointed out, by entry of the salt or by renewal of the osmotically active material, a reaction follows such as is not possible in a wilted plant. Further, it is difficult for a wilted plant to retain definitely a reduced quantity of water ; it will either re-absorb water and recover or give off more and die. Yet there are many plants which may be preserved in the plasmolysed condition for a long time without death taking place. Algae, for example, may be so treated and preserved alive for many weeks. No growth, however, is observable in them, though new cell-walls may be formed ; every plasmolysed cell in the long run dies. Each alteration in concentration or of the osmotic pressure of the external medium induces injury, and even the periodic changes which take place, for example, in the water in the estuary of a river, dependent on ebb and flow of tides, are such as few Algae can tolerate (Oltmanns, 1891).

It will be seen from these remarks that the rate of growth and the final size attained by every plant depend on the amount of water it contains, and this in turn depends on the relation subsisting between absorption from the soil and transpiration into the air. In addition to many other factors the amount of moisture in the air, as well as the quantity of water and salts in the soil, plays an important part. The minimum, optimum, and maximum for different plants, and even for individual organs of a single plant, vary greatly. The researches of Tucker and Seelhorst (1898) on the influence of water on the relationship between the roots and sub-aerial organs of the oat are of special interest. A limited amount of water in the soil excites active growth in the root ; it cannot, however, in spite of its great extent of absorbent surface, supply the aerial parts with sufficient water, and hence these remain small, the relation between the root and the whole plant being 1 : 7·4 while it reaches 1 : 16·6 when there is plenty of water in the soil. In this latter case the root remains small, *its* optimum being already exceeded. Thus between the root and the shoot there are appropriate correlations.

We may now turn to the effect of moist and dry air as factors in determining form in the plant. It is impossible for us to enter into detail on this question—a statement of the general results must suffice, because the material for discussion is so abundant. It has been shown that retardation and acceleration of transpiration very often form a self-regulating mechanism ; the plant in dry air develops adaptations for *reducing* transpiration, in moist air for *accelerating* it. The variability of the plant, and especially of the higher plant, has been shown to be far greater in this respect than any one would have imagined twenty years ago. These adaptations are seen not only in external form, but also in anatomical structure. Plants grown in damp atmospheres have longer internodes and petioles, and larger but thinner laminae. The weakly transpiring leaves of *Tropaeolum* are, according to Kohl (1886), five times

as large as those of plants grown in dry air and soil. Thus the outlines of organs become less irregular in damp atmospheres (KOHL, 1886), that is to say the projections from the leaf become less marked, the ribs on the stem tend to disappear, and the development of hairs becomes reduced. The anatomical differences are even more remarkable. Increased transpiration tends to thickening of the cuticle and induces the development of collenchyma and sclerenchyma, the vessels are larger and more numerous, and abundant palisade parenchyma is formed in the leaf. Critical researches are still required, however, to determine for us how far the observed results are to be put down simply to the difference in the *amount of water* present in the plant, and how far to the differences in the *rate of transpiration*; if the latter factor be the important one it will be necessary in the next place to find out whether the giving off of water as such acts as a stimulus, or whether the supply of nutrients which stand in close relation to transpiration is of greater significance.

When plants which normally live in dry regions are cultivated in a moist chamber very remarkable results are obtained. LOTHELIER (1893) found that the formation of thorns was inhibited in a very damp atmosphere; for example, *Berberis* developed leaves in place of thorns and *Ulex* developed ordinary leafy branches. GOEBEL (1898), who repeated these experiments, was not, however, able to confirm these results entirely; he was able to observe retardation, but not complete inhibition, of thorn formation. Still more remarkable results were obtained by BRENNER (1900) from succulents. Fig. 89 (p. 305) shows the habit of *Sempervivum assimile*, at *I* under normal conditions, and at *II* after lengthened culture in damp air. As transpiration becomes weak the elongation of the internodes destroys the radical rosette but later on a new rosette is formed. The leaves grow more vigorously on their upper sides and hence bend towards the ground in arches; at the same time they become markedly thinner. This diminution in thickness, the resolution of the rosette (which has been observed in other plants under the same conditions by WIESNER (1891)), and finally certain anatomical alterations, e. g. the bulging out of the epidermal cells, were correctly considered by BRENNER as adaptations to aid transpiration.

Many of the alterations in form and anatomical structure observed to take place in plants grown in damp air remind us of those which occur in plants kept in darkness. *Sempervivum assimile*, for example, loses its rosette form in darkness and its leaves are markedly smaller. Increase in dampness of the atmosphere is, however, very often associated in nature with diminution in light, and, conversely, strong insolation is often mostly associated with more active transpiration. In nature it is very rarely that we get a *single* factor determining the shape of the plant. If we find, for example, that subterranean branches are different anatomically and morphologically (COSTANTIN, 1883 and 1886) from aerial branches, we are led to believe the true reason lies not only in differences in illumination, but also in the amount of water present in the medium, and possibly even in the fact that such branches are in intimate contact with soil particles. Similarly, the characteristic structure of water-plants is concomitant not only with retardation of transpiration, but also with the changed relationship to light, oxygen, carbon-dioxide, &c. According to MACCALLUM (1902), the active cause of the aquatic form of *Proserpinaca palustris* is due entirely to retardation of transpiration; if transpiration be compensated by the osmotic absorption of a concentrated salt solution, typical aerial leaves arise. We await confirmation of these results. [The studies of BURNS (1904) show that this phenomenon is not so simple as it seems.] Similarly, *several* factors contribute to the formation of the alpine (BONNIER, 1895) and the halophytic (SCHIMPER, 1891; STAHL, 1894) types of plant life. It is impossible for us to enter into further detail here, so we will content ourselves with noting that the structure of the plant is not predetermined once and for all, but that it is capable of being *modified* by external conditions.

We have yet to glance at the effect of *other organisms* on plant shape. The plant can no more escape from the influence of such associations than it can from heat or gravity, since, wherever organisms occur, there we find a competition for space, for light, and for food. The result of this contest is that many of the organisms are killed off because the survivors have prevented them from obtaining the necessary supplies of food material or light. The action of the organism in these cases is only to induce chemical or physical alterations in the surroundings, and these, in their turn, bring about such effects as we have already made ourselves acquainted with. On the other hand, if an organism *injures* the plant, if it be partly eaten by an animal, for instance, changes occur in the plant body not merely in consequence of the injury as such, but as the result of the reaction of the plant to it. The direct effect of injury needs no elucidation—the indirect effects will be treated of in the next lecture. The relations existing between the plant and animal worlds are well known, such as those associated with the transference of pollen by insects and the phenomena seen in myrmecophilous plants. There can be no doubt that plants *are* adapted to the visits of insects, and *have* become altered in shape accordingly ; but this is a phylogenetic phenomenon on which as yet no experimental research has been carried out, so that we may omit any consideration of it.

In addition to the physico-chemical influences of the organism on the *environment* above referred to and the related effects of the presence of other organisms, there remains for consideration the *direct* influence of one organism on another, as when two organisms live together symbiotically or antibiotically. Here also the alterations in structure and appearance, often very remarkable, may be referred ultimately to chemical and mechanical factors, but it is impossible to treat of these problems comprehensively, because we do not know how these factors act individually, and also because in nature they emanate from the organisms themselves. We may, however, quote a few examples of the morphogenetic effects which arise from symbiosis or parasitism.

Variations from the normal plant shape are induced by the action of certain parasites, and known as galls. It is impossible to cite more than a fraction of the extensive literature on galls; the following references must therefore suffice :— Hofmeister, 1868 ; Goebel, 1898 ; Eckstein, 1891; Küster, 1903. Fungi are the most prominent causes of galls in plants, and in addition Bacteria (p. 238), Myxomycetes, and Algae ; parasites of higher rank are usually not counted as gall-producers, although they also may induce 'variations from the normal plant shape'. Among animals we take account first of all of gall-wasps and gnats, but there are other insects, as well as worms, which also produce galls. Let us consider first of all fungus-galls. The effect of the fungus on its substratum may be to destroy the immediate region affected or, in the long run, the whole plant. That this is due to the activity of a poison given off by the fungus is obvious, and there are cases known where the poison has been isolated, as, for example, *oxalic acid* (De Bary, 1884 ; Reinhardt, 1892). Such a radical procedure on the part of the fungus is, however, not to the purpose, since rapid growth of the fungus exposes it to injury by destroying its host-plant and finally itself. The behaviour of other Fungi, however, is much more to the point since they do not injure their host-plants at all or may even stimulate them to more vigorous growth. Many Uredineae, and also *Erysiphe guttata*, stimulate the formation of *chlorophyll* in the host ; the Synchytrideae cause the epidermal and neighbouring cells to grow vigorously in size, while others cause swelling of entire internodes, leaves, fruits, &c. Where such *hypertrophies* are induced we speak of genuine gall-formation. Anatomical investigation shows a great enlargement of the parenchyma cells in these hypertrophies and often also an increase in number of these, and the development in all of them of abundant protoplasm and starch. These alterations are all to the benefit of the fungus as supplying it with extra

nutritive material. This active development of parenchyma is accompanied by a reduction in the amount of sclerenchyma and collenchyma. It may be assumed that these galls are induced by some substances excreted by the fungus which operate in such a way as to stimulate growth in the host-plant just as many poisons do.

The *alterations in form* effected by other Fungi are even more peculiar than these comparatively simple hypertrophies. Thus the whole appearance of certain species of *Euphorbia* is altered by *Uromyces pisi*, and *Melampsorella cerastii* produces the well-known 'witch-brooms' on the silver fir, altering dorsiventral into radial shoots and perennial into annual leaves. Both of these forms of Uredineae ripen their aecidiospores on the metamorphosed plant, and these germinate on another host, producing in it no modification worth speaking of. Whether that be due to an alteration in the fungus or to peculiarities in the second host cannot at present be determined. Further examples of such modifications of form are given by *Peronospora violacea*, which alters the stamens of *Knautia arvensis* into petals forming a 'double flower', and by *Ustilago antherarum*, which stimulates growth in the otherwise dwarf stamens of the female flower of *Lychnis vespertina*, and so *apparently* brings about the formation of a hermaphrodite flower; the anthers are, however, filled with the reproductive organs of the fungus only and produce no pollen.

The greatest modifications produced by Fungi are the *new formations*, e. g. the spherical outgrowths of the alpine rose which remind one of *Cynips*-galls, but are really due to the attacks of *Exobasidium vaccinii*, and especially the witch-brooms induced by *Taphrina laurenciana*, that is to say, the adventitious shoots with abnormal leaves which arise from the foliage leaves of *Pteris quadriaurita*. [A very thorough study of the anatomy of fungus-galls has been carried out by GUTTENBERG (1905).]

The galls induced by insects are far more varied and, in extreme cases, more complicated than fungus-galls. We may note first of all cases where the organs of the plant attacked are altered into others, and where the organs develop normally but in unusual situations. *Livia juncorum* causes the alteration of foliage-leaves in species of *Juncus* into scale-leaves, and *Chermes* and *Lonchaea lasiophthalma* induce similar abnormalities in the spruce and in *Cynodon dactylon* (Fig. 94) respectively.

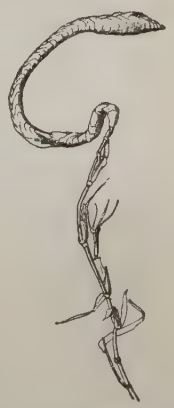


Fig. 94. *Lonchaea*-gall on *Cynodon dactylon*. About half natural size.

The transformation of floral-leaves into foliage-leaves was induced by PEYRITSCH (1882) by inoculating species of *Arabis* with aphides, and the same investigator was able to induce the formation of vegetative-shoots in the flowers of Cruciferae and Valerianaceae by inoculation with *Phytoptus*.

Other types of gall arise by local hypertrophy in otherwise unaltered organs. An example of this is seen in the bladder-galls of *Viburnum lantana* (Fig. 95), where we meet with an enlargement only of the cells already formed; usually, however, active cell division takes place along with excessive superficial growth or growth in thickness. Local *surface*-growth takes place on leaves in the common bladder-galls; local growth in *thickness* is illustrated by the very interesting *Cynips*-galls, which deserve more detailed treatment. These are interesting in the first place because they exhibit *specific* characters both as to form and size, which become more prominent the larger they grow, and because they exhibit peculiarities of internal structure which are unmistakably of the greatest importance, not to the plant, but to the insect causing the gall. We will, therefore, study a characteristic example of a *Cynips*-gall both from the point of view of

structure and of development, and will select for the purpose the leaf-galls so well known as occurring on the leaves of the oak, a detailed acquaintance with which we owe to the splendid researches of BEIJERINCK (1882).

The galls of *Dryophanta folii* are green spheres changing to red, from one to three cm. in diameter, attached to the ribs on the under side of the

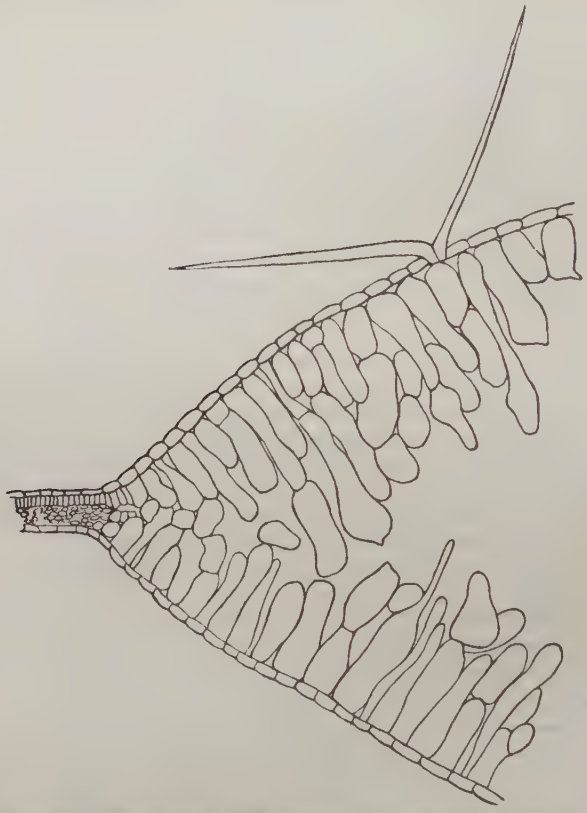


Fig. 95. Part of a transverse section through the bladder-gall of *Viburnum lantana*; a part of the unaltered leaf is seen on the left-hand side. After KÜSTER (Pathol. Pflanzenanat. Jena, 1903).

oak leaf. Beneath the green non-stomatiferous epidermis we find certain spherical cells, also green. These merge inwardly into a mesophyll with unusually large intercellular spaces, characterized by possessing abundant tannin. In a cavity in the interior, situated centrally, we find in autumn the insect surrounded by a shell of thick-walled parenchyma. It then gnaws for itself a canal out to the exterior, and breaks through in November at the onset of colder weather. All the insects hatched are female, and lay eggs without fertilization, a not infrequent phenomenon in the Insecta. The insect selects a resting bud at the base of the older branches to lay its eggs in; it bores through the bud-scales (Fig. 96, *I*) and lays its egg exactly on the apex of the growing point, fastening it there with a drop of slime. From the position where the egg is laid one may conclude that something more than a leaf-gall will arise. The egg in springtime becomes enclosed by the growing apex, and comes to lie in the middle of a mass of merismatic tissue of considerable size which has arisen from the growing point and its adjacent outgrowths (*III*). The gall then arises from the bud, and is in its full-grown state about 2 mm. thick and 4–5 mm. long. Its general appearance is represented at Fig. 96, *IV*. We

notice an apical elongation—the gall proper—and basally a number of unaltered bud-scales. A longitudinal section through a young stage is shown at Fig 96, *V*. In the centre lies a space containing the larva ; round it there is a layer of cells, characterized by their large nuclei and abundant proteid and fatty contents. This we may term the nutritive layer, for on it the larva feeds. The whole of the space between the nutritive layer and the papillate epidermis is composed of greatly thickened amyliferous cells which remain in the ripe gall. Finally vascular bundles branch abundantly through the cortex of the gall from below upwards.

At the beginning of June the insects escape ; this time some are male, some are female. The latter are like the leaf-wasps but are much smaller. These insects were known by the name of *Spathegaster taschenbergi* before their relation to *Dryophanta folii* was known, and hence even yet the galls are spoken of as 'Taschenberg galls'. The female 'spathegasters' after fertilization betake themselves to the under side of immature leaves, bore deeply into one of the larger veins with their ovipositors and lay eggs in the canal so formed. A gall then arises from the phloem of the neighbouring vascular bundle which soon bursts the cortex (Fig. 97, *I*), and in a central space the young larva develops after being released from the egg-shell (*II*). The gall developing thus endogenously, like a root, grows until it becomes an externally visible sphere, fastened only by a short peduncle to the inner edge of the leaf-vein. It shows a differentiation of tissue (*III*) into three concentric layers. The innermost forms a nutritive layer for the larva, then follows a sclerotic layer, and externally a thick cortical region plentifully supplied with vascular bundles. At this stage, however, the gall is by no means fully formed. Later on the sclerotic layer and the cortex increase markedly, and solitary thin-walled cells, as well as the thinnest-walled sclerotic cells, grow greatly and become at the same time filled with reserves. BEIJERINCK terms this the secondary nutritive layer, since it, like the primary layers, serves for the support of the larva ; the largest cells are always to be found just where the larva feeds.

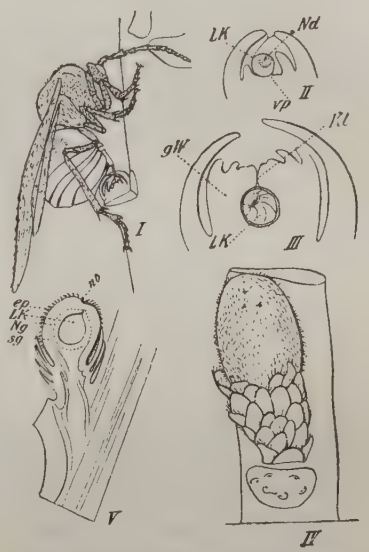


Fig. 96. Galls of *Spathegaster taschenbergi*. After BEIJERINCK (1882). *I*, leaf-wasp laying an egg on a bud apex. *II*, growing point (*vp*) of a bud with larva, *LK*, and yolk, *Nd*. *III*, the same after enclosure of the egg ; *gW*, the investing tissue ; *LK*, larva ; *Kl*, cavity. *IV*, young gall in general view. *V*, young gall in longitudinal section ; *LK*, larval chamber ; *sg*, starch layer ; *ep*, epidermis.

Obviously the larva excretes something which acts as a stimulus to cell growth. This stimulus may be either chemical or mechanical, and the question comes to be what is the stimulus which induces the gall-formation as a whole. First of all it is certain that the wound caused by the puncture made by the insect need not be taken into account. Further, the movements of the larvae, looked at as mechanical stimuli, cannot be made answerable for the formation of the gall, since gall-formation commences while the larva is still completely enclosed in its egg-shell. Some definite substance must diffuse out from the larva which stimulates the cells to hypertrophy. In certain cases, e.g. in *Nematus*-galls on willows, according to BEIJERINCK (1888) the female during the process of laying the egg excretes a substance which induces gall-formation, so that small galls may develop without the development of a larva, but

W. MAGNUS (1903) was unable to confirm this. In the majority of cases the formation of the gall is entirely dependent on the *development of the larva*. It is quite unknown, however, how the *differentiation* of the tissues of the gall takes place, whether, for example, the formation of the nutritive layer depends on substances other than those which form the sclerotic layer.

In general, complicated galls, like those we have been considering, arise from *embryonic tissues*; these are at least more readily transformed than full-grown ones. We must leave undetermined the question whether, as BEIJERINCK holds, the gall actually arises from the *phloem* or whether the cambium takes part in the process. It is important to note that the gall-formation arises often from quite uninjured cells, so that the stimulating substance is obviously *diffusible*. In favour of this view is the fact that galls are in general concentric to the larva and that the action of the stimulus appears to cease at a certain distance from the centre. HOFMEISTER (1868) also held that the cause of the gall was a fluid excreted by the insect.

The gall-insect thus provides definite chemical substances which act as stimuli, and the plant responds by forming a gall. These chemical stimuli produce different galls in different plants; thus the gall produced by *Cecidomyia artemisiae* on *Artemisia campestris* differs from that on *A. scoparia*. From this

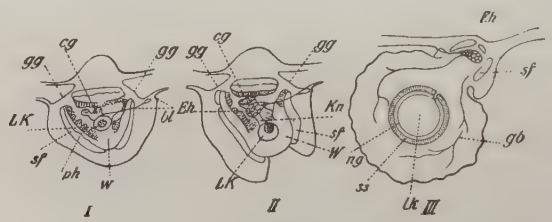


Fig. 97. Development of the gall of *Dryophanta folii* Transverse section through the midrib of the oak. After BEIJERINCK (1882). *I*, formation of the gall swelling (*W*) from the phloem. *II*, bursting of the cortex. *III*, young gall, almost complete; *gg*, boundaries of the assimilatory tissue of the leaf; *sf*, sclerenchyma; *ph*, phloem; *cg*, vascular bundles arising from the midrib; *W*, gall swelling; *Kn*, canal; *LK*, body of the larva; *Eh*, egg cavity; *lk*, larval chamber; *ng*, nutrient layer; *ss*, sclerotic layer.

it follows that not only the insect but the plant also plays an important rôle in gall-formation. It is all the more remarkable that the *plant* itself makes no use of the galls.

On the other hand the use of the gall-structure to the *animal* is obvious The differentiation of tissues seen in *Dryophanta*-galls occur also in others; tissues, especially nutritive ones, serving mechanically or chemically as a protection to the insect, are common. Frequently cell-forms appear which are entirely wanting not only in the normal plant but in any of its near relatives. We also find other adaptations whose functions in relation to the *parasite* are even more obvious, such, for example, as the formation of lids to the galls of *Cecidoses eremita* (KERNER, 1891, II, 526, Fig. 5). The inhabitants of the galls make use of their hosts to the fullest extent without giving any equivalent in return. The plant, too, makes no attempt to get rid of its parasite, it renders up willingly all the material wanted by it, it even builds it a house, and, in short, treats it as though it were one of its own organs. We may, therefore, conclude that the plant cannot differentiate between the materials formed by the larvae and by its own members, and that, in the normal course of development also, the *material* influences of one part on another must be of great moment.

Relationships of another but equally interesting type are to be met with, finally, in *lichens*, which we may cite as an example of symbiosis. As has already been mentioned (p. 243), each of the organisms which combine to form the association obtains some advantage from the union, but

it is not always easy to prove this. We have already gained some acquaintance with the *stimulating effects* of genuine parasites, but possibly the algal constituents of lichens do not *always* benefit in that way; at all events, we notice one injurious result, viz. that they do not produce fruit when so united. As to the form of the federation we may distinguish important differences. In many lichens, e.g. *Ephebe*, the alga is the predominant partner and the lichen has essentially the appearance of the alga without the fungus. *Cora*, on the other hand, one of the Hymenolichenes, has exactly the appearance of the constituent fungus, one of the Telephoreae. In the majority of cases the symbiotic union results in an entirely new form, with all the characters of a single independent organism. In these cases obviously each organism influences the other, and sometimes the one, sometimes the other, partner is predominant. According to MÖLLER'S (1893) researches, this is the case with *Dictyonema*, a lichen the fungal constituents of which is the same species of Telephoreae which occur in *Cora*. If the alga be a member of the Chroococcaceae, the lichen *Cora* is the result, but a *Dictyonema* if it be a *Scytonema*. The filaments of *Scytonema*, however, are much more vigorous than the unicellular Chroococcaceae, and thus produce more arbitrary growth forms. The alga and the fungus carry on a sort of struggle to determine the ultimate form of the compound organism. If the lichen be developed in the air the fungus is the dominant partner (true *Dictyonema* form), but if it be transferred to a solid support, the alga gains the upper hand and it alone determines what the form of the compound organism shall be (*Laudatea* form), and the fungus becomes merely an accompaniment (MÖLLER, 1893). The fungus can also live apart from the alga as a member of the Telephoreae but may, when the suitable alga is met with, assume in the course of its development either the *Cora*, *Dictyonema*, or *Laudatea* form.

Bibliography to Lecture XXV.

[BALL. 1903. Jahrb. f. wiss. Bot. 39, 305.]
DE BARY. 1884. Morphologie u. Biologie d. Pilze. Leipzig.
BENECKE, W. 1903. Bot. Ztg. 61, 19.
BEIJERINCK. 1882. Beobachtungen üb. die ersten Entwicklungsstadien bei Cynipidengallen. Amsterdam.
BEIJERINCK. 1888. Bot. Ztg. 46, 1.
BONNIER. 1895. Annales Sc. nat. VII, 20, 217.
BRENNER. 1900. Flora, 87, 387.
BURCK. 1900. Kon. Akad. van Wetensch. Amsterdam. Proc.
[BURNS. 1904. Annals of Botany, 18, 579.]
COSTANTIN. 1883, 1886. Annales Sc. nat. VI, 16, 5; VII, 1, 135.
ECKSTEIN. 1891. Pflanzengallen und Gallentiere. Leipzig.
ELFVING. 1880. Acta Soc. Fennicae, 12.
GOEBEL. 1898. Organographie, 1. Jena.
[GUTTENBERG. 1905. Beitr. z. phys. Anatomie der Pilzgallen. Leipzig.]
HARTIG. 1901. Holzuntersuchungen. Altes u. Neues. Berlin.
HEGLER. 1893. Cohn's Beitr. z. Biologie, 6, 383.
HEINRICHER. 1896. Ber. naturw. Verein. Innsbruck, 22.
[HERING. 1904. Jahrb. f. wiss. Bot. 40, 499.]
HOFMEISTER. 1868. Allgem. Morphologie d. Gewächse. Leipzig.
[KELLER. 1904. Einfl. von Belastung, etc. auf Ausbild. d. Gewebes in Fruchtstielen. Diss. Kiel.]
KERNER. 1891. Pflanzenleben. Leipzig and Vienna.
KNY. 1901. Jahrb. f. wiss. Bot. 37, 55.
KOHL. 1886. Die Transpiration. Braunschweig.
KÜSTER. 1903. Patholog. Pflanzenanatomie. Jena.
LENGERCKEN. 1885. Bot. Ztg. 43, 337.
LOTHELIER. 1893. Revue gén. de Bot. 5, 480.
LÜPKE. 1888. Landwirtsch. Jahrb. 17, 912.
MACCALLUM. 1902. Bot. Gaz. 34, 193. Abstr. Bot. Ztg. 1903, p. 69.

MAGNUS, W. 1903. Ber. d. bot. Gesell. 21, 129.
MÖLLER. 1893. Flora, 77, 254.
MOTTIER. 1899. Annals of Botany, 13, 346.
NABOKICH. 1901. Ber. d. bot. Gesell. 19, 222.
NABOKICH. 1902. Beihefte Bot. Centrbl. 13, 272.
[NIKITINSKY. 1904. Jahrb. f. wiss. Bot. 40, 1.]
NOLL. 1900. Landw. Jahrbücher, 29, 361.
NOLL. 1901. Sitzungsber. niederrh. Gesell.
OLTMANNS. 1891. Sitzungsber. Berliner Akad. d. Wiss.
[PERODKO. 1904 Jahrb. f. wiss. Bot. 41, 1.]
PEYRITSCH. 1882. Jahrb. f. wiss. Bot. 13, 1.
PFEFFER. 1893. Druck u. Arbeitsleistung. (Abh. Kgl. Gesell. Leipzig, 20, 233.)
PULST. 1902. Jahrb. f. wiss. Bot. 37, 205.
REINHARDT. 1892. Jahrb. f. wiss. Bot. 23, 479.
RICHTER. 1894. Flora, 78, 423.
SCHIMPER. 1891. Indo-malayische Strandflora. Jena.
SCHWARZ, F. 1881. Unters. Bot. Inst. Tübingen, 1, 53.
SCHWARZ, F. 1883. Ibid. 1, 135.
STAHL. 1894. Bot. Ztg. 52, 117.
TUCKER and SEELHORST. 1898. Journal f. Landw. Reviewed in Biedermann's Jahresber. 28, 269.
VÖCHTING. 1878. Die Organbildung, 1. Bonn.
[WIEDERSHEIM. 1902. Jahrb. f. wiss. Bot. 38, 41.]
WIELER. 1883. Unters. aus d. bot. Inst. Tübingen, 1, 189.
WIELER. 1901. Ber. d. bot. Gesell. 19, 366.
WIESNER. 1891. Ber. d. bot. Gesell. 9, 46.
WIESNER. 1895. Ibid. 13, 481.
WIESNER. 1896. Ibid. 14, 180.
WORTMANN. 1881. Bot. Ztg. 39, 368.

LECTURE XXVI

CORRELATIONS

SINCE, as we pointed out at the end of the last lecture, an alga living symbiotically with a fungus can markedly influence the mode of growth of the other member of the partnership, although a give-and-take of soluble materials is almost the only relation that subsists between them, and, further, since an insect, which presumably operates only by excreting some chemical substance, can induce the formation of galls, and, though a *foreign* organism, bring about far-reaching modifications in plant form, it follows that individual organs, bound together by intercellular protoplasmic threads into one organic unity, must also influence each other in a very marked manner. Such relationships of plant-organs, which may be termed 'growth-correlations' (GOEBEL, 1880), are very generally met with and claim our attention here. They stand on the border-line between internal and external growth-factors, for if we regard the individual cell or growing point as relatively independent, influences exerted on it by other cells or other growing points of the same plant may be regarded as *external* influences, since all parts of the plant, save that immediately under consideration, are in the position of an environment to it. If, however, we look upon the entire plant as a single unit then the action of one part on another must be considered as that of an *internal* factor.

The cells of *Spirogyra*, which are all alike and which all perform the same functions, do not apparently influence each other's growth, and it would appear that, so far as the welfare of the individual cell is concerned, it is immaterial whether they be united to each other or not.

If, however, different cells or, speaking more generally, different organs of a plant-body differ in structure and function, then they necessarily influence

each other, inasmuch as the performance of a definite function in one organ is essential to the carrying out of other functions in other organs, although these organs may be quite capable in themselves of performing such functions. In normal ontogeny each functional member assumes a definite shape, and we might readily suppose that it *could* not assume any other form. As a matter of fact, however, we should be more correct in asserting that *every* organ arising at the growing point may develop in a *variety of ways ;* the fact that it is forced to assume a definite direction of development depends only on its relations to other parts. Did no such regulation of the development of parts exist and if every cell or every mass of embryonic tissue gave rise to such structure as it was inherently capable of producing, the resulting plant would be no longer an *organism* but merely a chaotic mass of living substance. ' Harmonious development ' is possible only if correlations exist, and to gain some acquaintance with examples of these correlations must be our first task, thus giving us the opportunity of considering a whole series of phenomena which we have hitherto only imperfectly studied. In only a few cases is it possible to draw conclusions as to correlation from *observation* only. Berthold (1882) has found that the lateral branches of many Algae may give rise to new outgrowths at their *bases* on the *convex* side turned away from the chief axis, the effect of the chief shoot being to cause the normally radial branches to become locally dorsiventral. Similar observations have been made on twigs of *Cupressus* ; the lateral branches bear more numerous and larger proliferations on the side towards the apex, although their disposition should be basiscopic. Occasional *anomalies* may also be observed. Thus De Vries (1891) records the case of a flower-stalk of *Pelargonium* which formed a leaf-bud, and which, instead of dying off after the bud had opened, remained in existence for several years, and by vigorous secondary growth formed a woody cylinder like an ordinary stem. As a general rule the flower-stalk forms no cambium nor has it any capacity for forming merismatic tissue ; it remains bare and no foliage-leaves arise on it ; in other words, a correlation exists between the development of foliage-leaves and the power of secondary growth in the stem. Similar examples worthy of notice often occur among malformations. *Experimental* studies on this subject are, however, of more importance and more pertinent.

From among such experimental researches we may select those which aim at observing the effect on the rest of a plant of the *removal* of one of its organs. This experiment necessitates, in the first instance, the formation of a wound which the plant endeavours at once to cicatrize [Massart, 1898]. In the second place, the plant exhibits a capacity for replacing the organ which it has lost. We have here to deal with a reaction on the part of the plant to *external* mechanical stimuli, such as we have described in the last lecture, but these processes become intelligible here for the first time in view of the light shed on them by a study of correlations.

First of all a few words may be said as to the healing of wounds, and we shall take our examples from the higher plants exclusively. The healing takes place very differently according to the age and specific character of the tissue affected. The injured cells and also the other cells adjacent to them die off, but the layer of cells next below—if they still contain protoplasm—begin to react so as to develop tissue over the wound. Many of the parenchymatous cells, without actually growing, divide parallel to the surface of the wound, and their walls become cutinized and thus interpolate a corky layer between the dead and the living tissue. This reaction teaches us that fully-grown cells may still retain the power of division, but we must not regard the destruction of the connexion with neighbouring cells as the *only* factor inducing this reaction, for materials from the injured cells, or other changes taking place during the infliction of the injury, might act as a stimulus.

In addition to the simple healing of a wound by means of cork we meet in other cases with a more complex method, viz. the formation of *callus*. The cells which remain uninjured near the edge of the wound begin to grow actively and curve over it ; cell division begins and finally develops there a thin-walled large-celled tissue of irregular form to which the name of callus is given. All cells containing protoplasm and nuclei, even epidermal cells, seem capable of forming callus, but, naturally, young cells, and especially cambium cells, are more vigorous than full-grown cells. [This capacity for forming callus disappears in some cases early, in other cases late ; very rapidly, for example, in the cells of the root cortex (Massart, 1898 ; Simon, 1904).] Small wounds, such as pricks in leaves, may be completely occluded by callus alone, but in larger wounds a development of the cortical cells takes place as well, accompanied by suberization of the outermost callus cells. In this way a replacement of the epidermal cells is attained, always the first object aimed at. This is, however, by no means the only purpose of the formation of callus. Without attempting to offer a complete exposition of the subject it will be sufficient if we cite only a few examples here. Let us glance first at callus formation on cuttings. As is well known, gardeners propagate many plants by very simple means, viz. by cutting off twigs, placing them in wet sand, and keeping them warm. If the plant has the power of forming new roots under these conditions then propagation by cuttings is possible, otherwise not. Before roots are formed from the cutting there is formed at its lower cut end, imbedded in the sand, a callus, formed from the cambium, phloem, parenchyma, and pith, as shown in Fig. 98, *I*. It will be seen from the figure that the cambiogenic callus cells overlap each other and thus the callus tissue comes to form a hemispherical cushion on the cut surface of the slip (Fig. 98, *II*). Similarly other callus masses develop on the cut surface, and by their fusion form in the long run a single hemispherical mass of tissue. When the wound is quite covered, corky layers appear in the callus which enclose the lower ends of the vascular bundles, and separate them from the new tissue. On the outer surface of the callus also a corky layer has already appeared. Later on, at a certain distance from this layer, a cambium develops which unites with that of the cutting and proceeds to form secondary wood inwardly and secondary bast outwardly. In a one-year-old cutting, therefore, we find the secondary tissue arranged at the base from within outwards as in the stem and in direct continuity with it. We shall direct attention later on to the formation of roots from the base of the cutting ; these are not shown in the figure.

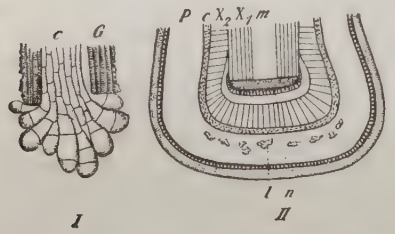


Fig. 98. Callus formation. *I*, longitudinal section through the base of a three days' old cutting of *Hibiscus*. Callus arising from the cambium, *c*; *G*, vessels. *II*, longitudinal section through the base of a one-year-old cutting ; *m*, medulla ; X_1, primary xylem ; X_2, secondary xylem ; *c*, cambium ; *p*, phloem and cortex ; *n*, cork ; *l*, sclerenchyma. After Stoll (1874, Bot. Ztg. 32, Pl. 12).

The callus in this case is able to form secondary tissues, but in other cases we find the callus capable of producing primary tissues. If, for example, we cut off the extreme apex of the growing point of a dicotyledonous root, the callus which arises is speedily able to produce a new growing point, which performs its functions quite normally and forms a new rootcap [Simon, 1904]. This property is, however, almost confined to the root ; it is seldom possessed by the stem (Peters, 1897).

As a rule, when an organ is removed its renewal does not take place from the callus exactly at the same place, but in the *neighbourhood*; this new organ may be already present previous to the infliction of the wound as a more or less

obvious rudiment, or it may be formed in the first instance from the callus. Many transitions occur between these types which are difficult to separate in practice. It is obvious, however, that the plant need not take the trouble to form an absolutely new organ if in the vicinity there exist already the rudiment of one identical with that it has lost. Thus, if the apex of a primary root be removed, the nearest lateral root takes on the function of chief root, and in the case of the stem there are plenty of buds which are only waiting their chance to take on the duty of forming the main axis. Generally speaking the basal lateral buds of a shoot are retarded in growth by the development of buds higher up; yet frequently in certain trees, such as the lime, the lateral buds high up inhibit the growth of the terminal one, and hence arises the 'sympodial' mode of growth of this tree.

When such 'reserve organs' (GOEBEL, 1903) are absent, a *fresh* growth of organs takes place from callus. This is of very common occurrence from the stumps of felled trees, where a cambiogenic callus produces numerous buds. Such a proliferation occurs in many plants on amputated portions of roots, and segments of potato tubers are especially capable of forming such shoots. Although in the majority of cases the origin of these buds is *endogenous*, as is normally the case in the root, there are also cases known where the renovation takes place from *epidermal* cells. The way in which plants of *Begonia* are propagated from leaves is well known. If leaves which have had their principal veins cut through be laid on wet sand a callus is formed at the end of each vein, in the formation of which the epidermal cells take part. Growth and division of *one* of the epidermal cells of the callus gives rise to a new shoot. It ought to be noted, however, that at a certain distance away from the callus uninjured epidermal cells may also give rise to new shoots, so that a normal epidermal cell can, without doubt, retain the power of developing buds. We therefore see that callus formation is by no means an *essential antecedent* to the development of shoots.

Roots also may arise after injuries to roots, stems, or leaves, either from rudiments of such already present in these situations or as entirely fresh formations. On the other hand, the plant has, as a general rule, no power of replacing leaves or parts of leaves. If the blade of a leaf be cut off, the petiole usually dies and is thrown off, and the stem also, when its apex is amputated, frequently dies off down to the node next below. HILDEBRAND (1898) has, however; drawn attention to a case of genuine renewal of the leaf-apex, and WINKLER (1902) and GOEBEL (1903) have recently investigated the matter more closely. If a leaf-blade of a young plant of *Cyclamen persicum* be cut off—only in a young plant is such an experiment successful—at a short distance from the wound a development of tissue takes place on both sides of the petiole, and this proceeds to form excrescences which, both in outward form and internal structure, must be described as new leaf-blades. [GOEBEL does not agree with WINKLER'S view that a genuine renovation of the leaf-blade occurs in this case; he holds that all that takes place is a continued growth of the leaf base previously correlatively inhibited. (Compare in this relation, as on all questions of regeneration, GOEBEL, 1905.)]

The instances we have quoted of the results of injury to the plant have made us acquainted with a number of cases of correlation. The plant possesses the power of replacing parts which have been lost, and thus cells, tissues, and higher members are formed anew, whose development by normal ontogenesis is impossible, since their connexion with the rest of the plant is destroyed. Regeneration appears not only in the *amputated* parts but also in the stocks whence the cuttings have been taken. We cannot avoid the conclusion that the capacity for manifesting vital activities of this sort exists in every cell containing protoplasm and that it is in general kept in abeyance only by the influence of related parts.

We gain a closer insight into the factors which determine plant formation when we examine more closely the situations where regeneration takes place. If

the apex of a shoot be removed the bud nearest to the wound proceeds to develop; if the apex of the root be cut off the nearest lateral root takes its place. As we have already seen, roots may appear on shoots and shoots on roots and even roots and shoots on leaves, and the position of these is governed by definite laws. A branch of willow bearing buds, if kept in a damp atmosphere, produces at its upper end leaf-buds only, and roots only at the lower end, and the size of the shoots increases the nearer they are to the upper end, and of the roots the nearer they are to the base. As VÖCHTING (1878) has shown, the distribution of organs, as well as their relative size, depends on external factors, such as moisture, gravity, and light, though not in the first instance conditioned by these factors. On the other hand, the branch has an inherent polarity of its own, and this may be influenced by external agents but cannot be completely overcome. In *Marchantia* and allied Hepaticae, which exhibit remarkable powers of regeneration (VÖCHTING, 1885), renovation always takes place at the apex of the older part; the very smallest segments of the thallus show a differentiation into two poles, and we can scarcely doubt that every cell possesses a distinct distal and proximal end. Indeed, VÖCHTING has also demonstrated the polarity of the individual cell in the higher plant (p. 333). We must remember in this relation that in the lower organisms the determination of what shall be base and what apex is frequently settled by external factors—in *Bryopsis*, for example, by light—but we must admit that the higher plants behave differently. In the phanerogamic embryo and the gemmae of *Marchantia* the differentiation of base and apex is already established, and depends entirely on internal factors. The phenomena of regeneration teach us, however, that the differentiation into shoot and root taking place after the first cell divisions in the embryo of Phanerogams does not indicate a separation of the protoplasm into two parts, one with root and the other with shoot characteristics, but that every individual cell must have permanently the capacity of forming *both* kinds of organ. Only the continuous mutual influence of the parts can condition the formation of a shoot on the one hand or a root on the other. It need scarcely be mentioned that the root behaves in principle just like a shoot, producing shoots at its base and roots at its apex; further, that the polarity in regenerative processes exhibits itself, very frequently at least, in the leaf, inasmuch as shoots arise on its morphological upper side and roots from its under side. Since this polarity is induced only by correlation, we need not wonder that, in certain cases, it becomes obliterated where the growing point of the root throws off its cap and produces leaves, in *Neottia* and *Anthurium*, for instance, apparently without any external interference, and in *Ophioglossum*, after separation of the ends of the roots. (For literature see GOEBEL, 1898–1901, p. 435.)

The simplest cases of correlative influence are *quantitative* in their nature, *one* organ determines to what *extent* another may develop ('compensation'; GOEBEL, 1884, 1893–5). In the last-mentioned examples, however, we have to deal with *qualitative* changes, and a few further examples of these may be given here. We may deal first with GOEBEL'S researches on bud-scales (1880). By removing the foliage-leaves at an appropriate time of the year it is possible to inhibit the formation of bud-scales and to transform their rudiments into foliage-leaves, and it is possible to bring about the change of subterranean buds into leafy shoots, as in the potato and many rhizomes, by removal of the main leafy shoot. We shall obtain a better conception of this phenomenon in the next lecture.

We may next select the spruce as an example of a far-reaching qualitative correlation between the main shoot and lateral buds. The main axis is radial and grows upright, the lateral shoots are dorsiventral and grow obliquely upwards. It we cut off the main shoot one (or often more) of the lateral shoots highest up the stem grows round as nearly as possible at right angles, and becomes radial in structure. It may be assumed that the dorsiventrality of many lateral

branches and of laterally-placed flowers is also due to the correlative influences of the main axis, and in this relation it should be remembered that normally dorsiventral flowers, such as those of Orchidaceae and Scrophulariaceae, generally become radial if they once become terminal (peloria).

We have now become acquainted with numerous correlations among phenomena of regeneration appearing after the infliction of wounds, but these regenerations are not the first or only consequences of injury. If a leaf-blade be cut off serious disturbances must ensue in the petiole and the portion of the stem immediately associated with it, which express themselves in reactions easily recognized. Such reactions, consisting in the throwing off of the petiole in the case of the leaf which has lost its lamina or the stem which has lost its leaves, have already been referred to. If, however, all the leaves are not removed from the stem there is no reason why the shoot should die off; on the other hand, it no longer develops conducting tissue to the removed leaves. There is also a relation between the leaf and the leaf-base on the stem, which may be studied very readily in the epicotyl of *Phaseolus multiflorus*. If we remove one of the two young primary leaves at the first node of the epicotyl and at the same time carefully cut off the stem apex (whose further development would complicate the experiment) we see a marked reduction in the diameter of the vascular elements on one side of the epicotyl, from the base to the apex, while those on the side on which the leaf still remains are developed normally and also show indications of secondary growth (Jost, 1891).

The conclusion to be drawn from this experiment is undoubtedly that the result is not due to the *injury* but to a suspension of function, and especially of the growth in the leaf. This is evidenced by the fact that the same stem-structure may be induced if the leaf be not cut off but only prevented from further growth by enclosure in plaster of Paris. In other cases also the removal of an organ is followed by purely *mechanical* growth retardation. Thus Hering (1896) has shown that the cotyledon of *Streptocarpus*, which normally does not develop, may be made to do so either by cutting off the one which does, or by enclosing it in plaster of Paris; Winkler also showed that leaf-regeneration could be induced in *Cyclamen* when he enclosed the leaves in plaster of Paris without injuring them. Since in this case we are dealing with fully-developed organs the plaster could not have acted as in *Streptocarpus* or *Phaseolus* by retarding growth, but rather by inducing a cessation of the *function* of the organ. He obtained similar results by using instead shells of collodium and shellac. We thus arrive at the conviction, which, however, still demands experimental confirmation in many points, that regenerations may be induced not only by removal of an organ but also by rendering it inactive. [As to the various factors concerned in regeneration compare Klebs (1903), Goebel (1905), and MacCallum (1905).] In the case of the plant it is difficult to distinguish whether an organ is removed or merely has its functional activity retarded.

Organs which are not functional are usually abstricted, as we have already seen in the case of petioles and stem-bases. Intact foliage-leaves in sensitive plants, e.g. *Mimosa*, are rapidly thrown off if they be prevented from assimilating by withdrawal of carbon-dioxide or by being kept in darkness (Vöchting, 1891; Jost, 1895). Certainly all plants are not so sensitive; evergreen leaves, for instance, which live the whole winter through without manifesting any activity, may be kept in the dark for months without dropping off.

We arrive at an exactly opposite result to that which we obtain with leaves prevented from assimilating, if organs which have lost their original function and are, in consequence, dying, take on new functions. Thus Vöchting (1887) was able by employing special artificial means to cause tubers of a potato which, owing to germination, had dried up and were dying off to remain alive for a year more, and he obtained even better results with *Oxalis crassicaulis* (1899), a plant

which may be compared with the potato in all essential points of structure. Normally the tubers germinating underground form several leafy shoots which root at the base and become independent after the exhaustion of the parent tuber. If the tubers be planted in springtime with only their lower ends in the soil, the *tubers* themselves form roots while the leafy shoots arising at their upper ends form none. The tuber thus is interpolated between the root and the shoot, and it has therefore to perform not only the function of a storehouse of reserves but also of a *conductor* of materials absorbed from the soil. It remains alive in this condition the whole summer, grows considerably in thickness, and develops cellular elements which are foreign to its nature but which are characteristic of the normal stem, viz. large vessels, sclerenchyma, and wood parenchyma. The need for elements to carry out the functions of conduction, support, and storage acts here, as in cases of regeneration, as a *stimulus* which induces the satisfying of this demand.

We owe to VÖCHTING (1899) also a large number of experiments in which tuberous plants were prevented from forming storage tissue. It is interesting to note that these plants then proceed to deposit their reserves in other organs and that these organs assume in consequence an entirely different structure and function. Under normal conditions all phenomena of this kind are not to be traced to the action of correlation. Two examples may be cited. If offsets of *Oxalis crassicaulis* laden with reserves be cut off and kept in a moist chamber they form normal tubers at their apices, but if all the growing points be removed a tuber is formed all the same either by cellular increase at one or two internodes or by the swelling of the scale-leaves. In this case a *completely*-developed organ has taken on a new function and shape, but such cases are rare. (Compare WINKLER, 1902, Ber. d. bot. Gesell. 20, 500, where in the case described the factors in the process are unknown). Both structures are possibly without significance in this plant because it neither possesses tubers nor can it form them.

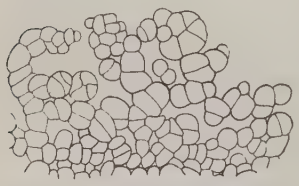


Fig. 99. Callus growth on the cut surface of beetroot. × 50. After VÖCHTING, 1892.

Boussingaultia baselloides, which develops normal subterranean stem tubers, is even more plastic. Such tubers may be induced to develop on every foliage-bud if the stem be treated as a cutting, and the axillary-bud be kept in the dark. If a cutting be treated so that its base, free of buds, is placed in the earth a large tuber arises at the basal end of the axis from the callus, which lives throughout the year without being able to produce a growing point. We need not enter into a discussion here as to the varied histological alterations which take place in the stem; we need only note that roots swell into tubers since the plant has no main axis in which to deposit its reserves. This takes place when leaves are used as cuttings. All plants are probably not so variable, still VÖCHTING'S researches have made us acquainted with numerous interesting facts, into which, however, we cannot enter further.

We have as yet only dealt with correlations which we may study by the simple experimental method of comparison of the results of *removing* or *inhibiting* the *functional* activity of organs. We may now consider a third method of demonstrating correlations, long known practically, and especially illustrated by VÖCHTING'S researches, viz. *transplantation* or artificial budding.

VÖCHTING (1892) has made a thorough study of the simpler forms of transplantation. He cut out a cube of beet and then replaced it in the original wound. A rapid healing of the wound took place by appropriate fusions, the cells which were not injured by the cut beginning to swell out, bud (Fig. 99), and grow together where they touched each other (Fig. 100, *III*). New

vascular bundles originated, connecting the portion inserted and the main root, and after a short time it was possible to determine only at certain places (*gg* in Fig. 100, *I*) where the transplantation had occurred and to observe there a demarcation between the graft and the stock. Similarly we may cut out a cortical block from a branch and replace it, allowing healing to occur. The amalgamation takes place if a sufficient number of protoplasmic cells capable of growing be present ; if these be absent, as in old wood, amalgamation is impossible ; if these cells are present only in one region, e.g. in cambium, a local amalgamation alone is possible.

If now the part cut out be inserted into the wound with *different* orientation, if, for example, it be turned inside out or upside down, amalgamation still takes place; but after a longer or shorter time a pathological condition ensues, for there develops a swelling which in extreme cases may bring about the death of the plant. The factor concerned in the tumour is *polarity*, which is functional in every cell and not only longitudinally but also radially. [MIEHE (1905) has demonstrated this polarity in individual cells of *Cladophora* in a very beautiful manner.] If we compare a plug of beet inserted in the orthodox manner (Fig. 100, *I*) with one inserted

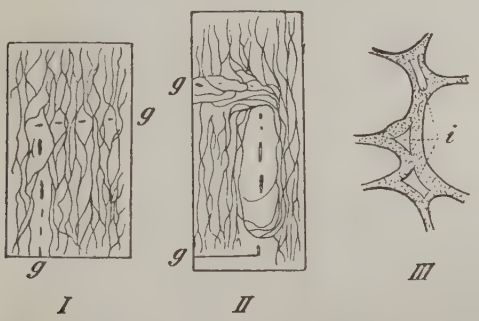


Fig. 100. Mode of growth of transplanted portion of beet. *I*, tangential section through the upper end of a portion of tissue inserted in the normal orientation. The limits of the tissue are indicated by the dotted lines *gg*. The longitudinal lines indicate vessels. *II*, a similar section of an inverted graft. The vascular bundles are developing almost entirely in the long axis ; *I* and *II* about natural size. *III*, individual cells from the area of fusion ; *i*, intercellular spaces and thickened cell-walls. × about 350. After VÖCHTING, 1892.

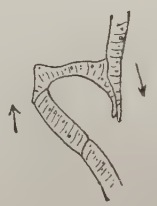


Fig. 101. Two vessels from *Cydonia japonica*. One is united to the other by an intermediate curved vessel. × 120. After VÖCHTING, 1892.

upside down (Fig. 100, *II*) we see that in the latter case the continuity of the vascular system is destroyed, the polarity of the vessels is altered in so far that their basal ends are unable to unite with the distal ends of those already present in the stock. The new formations attempt to bend round and lay themselves *sideways* against older vessels so that they orientate themselves in the same direction. Fig. 101 shows more exactly the mode of union of the vessels, the polarity of which is indicated by arrows. By no means all cells are able to effect an accurate junction ; the disturbance caused by the severance is permanent, and hence arises an active growth leading to a hypertrophy, suggestive of the effect produced by the attack of a parasite.

Similar results are produced when we plant a piece of tissue cut out of a certain region in another situation. It is possible to transplant in the same way a portion of tissue on another individual of the same species or on another species. Only experiments which aim at transplanting a portion of tissue bearing one or more growing points are of especial interest. Such transplantations are frequently carried out in gardening operations, as when budding or grafting of small twigs is resorted to. The part transplanted is spoken of as the graft or 'scion', and that to which the graft is attached as the 'stock'. In

grafting the scion has the form of a disk of cortex subtending a bud, carefully separated from the wood, and which is squeezed into the stock, close against the cambium, after the lifting up of two flaps of cortex. The cambium of the scion unites with the cambium of the stock and thereafter the bud develops. Of the numerous methods of budding we need select only one, viz. budding by clefting. The end of the stock is split longitudinally and the wedge-shaped base of the scion is sunk in the cleft. In this, as in the previous case, a good junction must be obtained if a fusion is to result.

We may, as we have already seen, transplant the scion on to another species, still it is impossible to graft *any* plant on another, for a certain degree of relationship between scion and stock is requisite, although the capacity for amalgamation runs by no means parallel with systematic relationship. Apples and pears, for example, graft badly on each other, although they belong to the same genus, while pears graft very readily on the quince, which is placed in another genus. Similarly potatoes graft more readily on *Datura* and *Physalis* than on many species of *Solanum*. We are able apparently to graft easily all other species of Cactaceae on *Peireskia aculeata*, while other species of *Peireskia* graft badly on this species. We must take cognisance of all these facts, although we may not be able to explain them. The point of interest for us, however, in these cases of transplantation lies in the numerous correlations which we may eludicate by their means. The interrelations between two species thus fused together must operate in the same way as between two parts of *one* plant since the union is a complete one, for STRASBURGER (1901) was able to prove a fusion of the protoplasm of the stock and scion.

The interactions which take place between scion and stock may be shown in the first place to be purely *quantitative*. There are plants which develop better as scions on other species than on the same species, e.g. *Physalis* on *Solanum*, *Arabis albida* on *Brassica oleracea*, *Solanum dulcamara* on *Lycopersicum*. On the other hand, the development of the scion may be retarded by the stock, and since retardation of vegetative growth is usually accompanied by increase in flower-formation, grafting is for this reason frequently resorted to in fruit-tree culture. Pears, for instance, which it is desired to cultivate as dwarfs, are grafted on the quince, and apples on *Malus paradisiaca* with the same object. Changes in mode of growth are often accompanied by a change in the duration of life, thus dwarf apple-trees, grafted on *Malus paradisiaca* grow only for fifteen to twenty-five years, while an ordinary apple-tree may attain an age of about 200 years. *Pistacia vera*, grown from a seedling, will live for 150 years, but if grafted on *Pistacia terebinthus*, for 200 years, if grafted on *Pistacia lentiscus*, for forty years only ; in the one case, therefore, the duration of its life is increased, in the other reduced. Annual plants cannot, as a rule, be made perennial by grafting, but LINDEMUTH (1901) has found that the annual *Modiola caroliniana* lived for 3½ years when *Abutilon thompsoni* was grafted on it, and similar results in other cases are extremely probable.

If we turn now to the special *qualitative* effect we must note first of all that as a rule (compare Lect. XXIX, p. 381) this effect does not go so far as to cause a modification of the *specific* characters of the two united plants, and on this knowledge rests the employment of the process of grafting in horticultural practice. There are other qualitative changes, however, which make their appearance, e.g. fruit-trees may be made, by grafting, to produce an increased yield of flowers and fruit. To VÖCHTING (1892) we owe a very interesting experiment on the qualitative effect of grafting. He showed that large-leaved shoots arise from the buds which spring from the base of the inflorescence of the beet in the second year if they be grafted on a one-year-old stock, inflorescences if on a two-year-old stock. These buds if left in their natural positions would, of course, have disappeared in autumn, but when transplanted they are stimulated to

further growth, and the way in which they grow depends on the stock. The external form is thus correlated with the life duration, which in one case lasts one year (i.e. the inflorescence), in the other two years.

Of equal interest is an experiment of LINDEMUTH (1901) on potatoes. The potato may be grafted successfully on *Datura*, for certain buds of the stock develop directly into horizontally placed aerial stola. In the course of the formation of these stola the disposition of the plant makes itself felt in the production of reserve stores which do not arise on the *Datura* stock. The stock, however, encourages an active vegetative growth, doubtless owing to its great capacity for forming roots, and the stola become not tubers but leafy shoots. If, however, the scion be grafted on such a stock of *Capsicum annuum*, which induces only feeble growth, the buds in question become tubers without any formation of stola.

We have spent too long over specific examples of correlations where attention has been paid more to the larger members (roots and shoots) and less to the tissues and cells. These latter also present us with abundant illustrations of correlation, although they have often not been *experimentally* established. To take one case only, we may recall the apposition of pits in cell-walls as an example of perfect correlation.

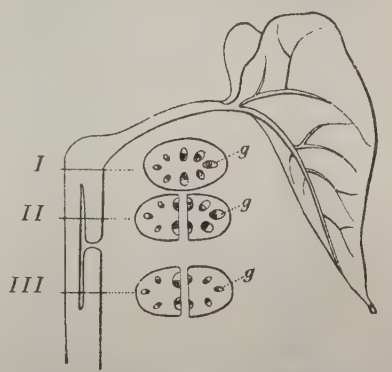


Fig. 102. Diagram of a plant of *Phaseolus*, on which only one primary leaf is allowed to develop. *I-III*, transverse sections at successive elevations; *g*, vascular bundles.

We have now in conclusion to summarize our conception of the operation of correlative influences? In certain simple cases we may explain them by a reference to *nutritive factors*. If out of many buds only some develop we must not assume that the non-development of the others is directly due to a deficiency in nutriment, since there is as much nutrient there as is necessary to permit all the buds to *begin* to grow. But this would be extremely disadvantageous for the plant. Obviously there is here an adaptation of such a kind that no growth begins when nutrition is feeble, and the amount of nutritive material must act as a *stimulant* in some way not known. In complicated cases of correlation, such as the connexion between leaf and leaf-spur mentioned above (p. 330), we cannot avoid assuming the existence of complex stimuli, although we are unable to determine their nature. We must look more closely into this case and investigate how the stimulus may be transferred from the leaf to the next lower internode. It is easily shown, in the first place, that the development of the vascular bundle is quite independent of assimilation and the nutritive processes connected with it, since the experiment may be carried out equally well in light and darkness. We might further believe that the *functional* activity of the vascular bundle on the side of the leaf—the passage of water through the vessels and of plastic organic materials through the phloem—provides the stimulus from which follows the secondary thickening (DE VRIES, 1891). Such ' functional stimuli ' doubtless play a certain part in ontogenesis, but in the cases already cited they are not decisive factors at all events. We may, as shown in Fig. 102, split the epicotyl of *Phaseolus* longitudinally and then cut through transversely the part of the vascular system supplying the leaf left attached; the result is that the leaf-trace bundles passing backwards from the leaf develop quite as well as in the previous experiment although the nutritive stream from the cotyledons no longer flows in them nor

in their neighbourhood (JOST, 1893). We are here dealing with a quite special kind of effect—a stimulus action—which comes from the growing organ and propagates itself backwards. [Exceptions to this view are taken by MONTEMARTINI (1904).] We are unable to say, however, whether any definite chemical substance exudes from the leaf which acts as a stimulus. The similarity between the influence of the growing leaf and that due to the gall insect is worthy of note, and this likeness would tend to suggest that we are dealing in this case also with a chemical stimulus. Doubtless stimuli such as we meet with in *Phaseolus* may be frequently met with, and especially in the relationships of secondary growth of the main axis to the development of lateral organs, relationships which are easily understood from a biological point of view, though from a causal aspect they are not intelligible.

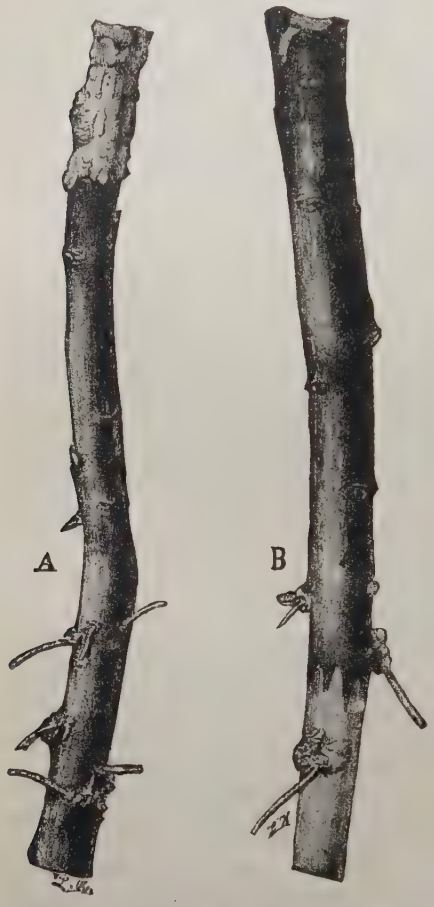


Fig. 103. Two cuttings of *Salix pentandra*, the upper ends freed from cork, the lower ends immersed in water, cultivated in a very moist atmosphere at a temperature of about 25°. *A*, in the normal position, with callus above and roots below; *B*, with roots developed from the (inverted) apical region.

An attempt has also been made to explain the phenomena of regeneration causally. But a causal explanation is naturally not provided by putting forward the appropriateness of the regeneration or by describing it as a 'tendency to completion of the body'. On the other hand, we more nearly arrive at a causal relationship after determining (compare p. 331) that the inhibition of the function of an organ may lead to regeneration as readily as its complete removal. There are two recent researches, one by GOEBEL (1903), the other by KLEBS (1903), which may be noted here as throwing light on the subject. We will select only one important item from KLEBS's paper. This author starts from the position—which must be accepted by all investigators—that every organ *laid down* in the plant must develop if all the conditions of its growth are fulfilled. He recognizes as one essential condition of the growth of the root a certain amount of water in the plant. Since a branch of a willow cut off from the main stem is able by developing roots to grow into an independent plant, that, according to KLEBS, can be accounted for only by an extra supply of water to the root primordia. Again, roots may be induced to shoot out at any place from willow twigs while they are still united with the plant if we permit the entry of sufficient water to their primordia by removal of the *cork*. Thus KLEBS was successful in getting roots to develop (Fig. 103) at the apex of twigs of willow, although up till then an upsetting of internal polarity had not been possible.

It is possible that the correlative inhibition of many buds by the chief

ones is due to the fact that too much water is withdrawn from them by the chief buds (compare WIESNER, 1889); but it is more probable that we have to consider in this relation not merely water but to look generally for the factors in such correlations in their relation to plastic materials. The case becomes much more difficult when we come to deal with *fundamental qualitative* changes; as to the factors concerned in such changes we shall have something to say in the next lecture.

From GOEBEL'S researches (1903) we may conclude that he was unable to refer the determination of the *position* of innovations to a polarity as yet by no means understood. According to GOEBEL, the *direction* of the *circulation of plasta* is of much greater consequence. Roots and stems show innovations at their apices and at other places towards which the stream of plasta generally trends; the leaf, however, shows regenerations at the base also, because the stream of assimilation products is directed backwards. With the object of supporting his view GOEBEL carried out a number of interesting experiments, but these were by no means exhaustive; much has yet to be done on the subject. Still, we are acquainted with many facts which are not in accord with GOEBEL'S conception. Basal innovations, for example, appear on the leaf and flower axis of *Achimenes* if these be treated as cuttings, and yet the course of migration of plasta in these organs is quite different (HANSEN, 1881).

Although we may be inclined, taking it altogether, to consider correlations as due to *plastic* influences of some sort yet we must not say that *mechanical* influences are entirely excluded; at least in the case of organs which touch each other such effects are quite possible. A distinction must be made between a *purely mechanical pressure* and a stimulus effect such as we referred to in a previous lecture on pressure, contact, &c. The present opportunity may be taken of saying a few words on mutual mechanical influences of organs.

Very frequently we meet with pressure at the growing point in the course of normal development, when the young members exhibit more vigorous growth than the bud-scales, or, to speak more generally, than the external foliar organs which enclose these young members. Many special bud arrangements are due to internal factors exclusively, others are due to space relationships. The crumpled petals of the poppy, for example, broaden out when the calyx is removed, but remain crumpled if again enclosed in a shell of plaster of Paris or other artificial calyx (ARNOLDI, 1900). Such unimportant and for the most part transitory effects are limited to the mutual pressure of organs at the growing point; only rarely are they seen in full-grown organs, as in the inwardly directed pressure of outer leaves of *Agave* on inner ones. As HOFMEISTER has shown (1868, p. 638) there is no case known where the formation of an organ is *essentially* conditioned by mechanical influences. What is true of formation is also true of position. A mechanical theory of leaf-arrangement has been established by SCHWENDENER (1878), and elaborated still farther by his pupils; he believed that *mechanical* relationships, especially the distribution of pressure at the growing point, determine the point of origin of new organs, and further that mutual pressure of organs may cause alteration in the primary arrangement. Alterations in position, such as those SCHWENDENER seeks to explain, are, however, nowhere observed, and they would also be absolutely unintelligible (SCHWENDENER, 1883, &c.; SCHUMANN, 1899; JOST, 1899, &c.).

Evidence in support of the assertion that the primordia of organs are conditioned by pressure is as yet entirely wanting. In individual cases, e.g. the formation of organs in axillary buds, the mechanical theory, although not proved, appears to us to be simple and clear. Since a pressure may frequently be produced on opposite sides of the growing point of the axillary bud by the subtending leaf and the axis, and if new leaves arise at the point of least pressure, they must appear laterally; but the relationships of any growing point bearing

the leaf-primordia are not so obvious as this, and they are certainly not 'mechanical'. As to the distribution of pressure on the cone above the youngest leaf-rudiments we know nothing. Recently LEISERING (1902), a pupil of SCHWENDENER, has directly proved that in these cases there is no *external pressure* exerted on the growing point. When LEISERING has recourse to internal tensions induced by the base of the projecting leaf, the theory is placed in a position where science surrenders the field to imagination. Any detailed criticism of the mechanical theory need not be entered into here. (Compare SCHWENDENER, 1883, &c.; VÖCHTING, 1894 and 1902; WINKLER, 1901 and 1903.) We need only add that in our opinion pressure has practically no significance as regards the arrangement of organs. [Compare FALKENBERG (1901, 41), and BERTHOLD (1904, 133).]

In those cases also, such as axillary buds, where there is nothing against the acceptance of the existence of pressure on the growing point, it is still an arbitrary proceeding to regard this pressure as the *only adequate* factor and to ignore all the other relations of the organs, which *must* exist on the analogy of the numerous examples of correlation known. In addition it may be mentioned that in not a few cases the same arrangement of lateral organs may be observed when these are placed at a distance for those next older, where contact is impossible (compare Fig. 65, p. 274), and where LEISERING'S internal tensions cannot exist. If, however, in such well-established cases contact plays no part it is not unnatural to suspect that it plays no part in others also.

Further, the arrangement of organs is not always determined by *adjacent organs*; the arrangement of lateral roots is known to be determined by internal anatomical structure, and it may be imagined that at the growing point of the stem, and in flower-buds especially, similar conditions occur. In *Campanula media* the orientation of the carpels follows that of the *calyx*, and, according to the number of the whorls which are interpolated between the calyx and the carpels, the latter alternate with, or are opposite to, the stamens (EICHLER, Blütendiagramme, I, 295. How the carpels come to know the arrangement of the sepals is the puzzle.

It may be doubted whether the mechanical theory is in the first place *purely mechanical*, and in LEISERING'S most recent publication the possibility of a *stimulus effect* of the supposed pressure is somewhat more clearly suggested. That such activities may exist no one will deny, but proof of their existence at the growing point is not as yet forthcoming. Although we may be unwilling to accept the mechanical theory of leaf arrangement we must at the same time admit that we have nothing better to put in its place, nor do we gain much by referring the arrangement of organs to correlations of growth in general terms. At all events it may be noted that there are certain regular arrangements of organs in the plant which must also be referred to correlation, such as the distribution of the xylem groups in the transverse section of the root and of sclerenchyma and assimilatory tissue in the cortex of the stem. No structures are fixed hereditarily once and for all, they are in the highest degree variable. Doubtless every cell in the periphery of the central cylinder of the root is capable of forming a vessel, just as every cell below the growing point is capable of forming a leaf; nevertheless, only certain cells at regular distances from each other become vessels and certain regions on the growing point become leaves.

The relations of the parts to each other and to the whole determine the path of development of each individual plant organ, it is not predetermined —that is the sum and substance of our interpretation of correlations.

It was remarked at the beginning of the present lecture that the phenomena of correlation could be regarded as internal or as external factors in plant formation. In the case of a complicated plant internal influences can scarcely act

directly on the growing parts, at all events on the growing point of the shoot; light, heat, chemical and physical influences of the material environment never affect the growing point itself. At the growing point or in its immediate neighbourhood it is decided what the organ shall develop into, and although external factors take part in this determination they can only do so *indirectly* through neighbouring older parts, that is, correlations always play a part.

Whether, however, the external factors operate *directly* on the part in process of formation or through the agency of older parts, they are always of the nature of *releasing* energies, and not the actual *causes of the formation* itself; that must *lie in the plant itself*, or more exactly in the *protoplasm*. Hence it is that plants have a cell structure and develop growing points, leaves, buds, roots, &c.; protoplasm is also responsible for the differences in the organs mentioned in different species. Although we may hesitate whether to consider the correlations as due to internal formative factors or not, we know sufficiently accurately that all organic formation in which protoplasm is concerned is conditioned by *internal* factors. In *this* domain lie the genuine problems of developmental physiology, from any insight into which we are as yet entirely excluded. It is as well to note that although we may establish the existence of one of the *external* factors in development we no more thereby attain an insight into the subject than we do into the structure of a complicated steam-engine by knowing who opened the valve. Since we learn nothing as to the mode of working of the machine by knowledge of this kind, it cannot be correct to designate the modest beginnings of developmental physiology as 'developmental mechanics'.

A short time since G. KLEBS (1903) classified the factors in plant form in three categories; he distinguished external and internal conditions of the resulting form and separated from the latter '*specific structure*', what we have designated as 'those in which protoplasm is concerned'. Specific structure is to a certain extent constant for each organism and on that depends everything else. The external world never acts on the specific structure *directly* but always on the internal *conditions*, on the quantity and quality of the substances present in the cell, and on the physical characters of the protoplasm, vacuole, cell-wall, &c.; and these internal conditions in turn affect specific structure. The internal conditions are to a certain degree open to investigation but the results of their actions are open only to a limited degree.

Although KLEBS's conclusions are, in principle, convincing, we still encounter difficulties in determining in special cases what depends on internal conditions and what on specific structure, and we have in the lectures which immediately follow, which were written before KLEBS's paper was published, distinguished only between internal and external factors; in separating these we make use of a sufficiently sharp but certainly superficial criterion. The external factors are such as may be referred to the action of gravity, light, &c., while the internal factors are those whose direct dependence on the external world cannot be demonstrated.

Bibliography to Lecture XXVI.

ARNOLDI. 1900. Flora, 87, 440.
BERTHOLD. 1882. Jahrb. f. wiss. Bot. 13, 569.
[BERTHOLD. 1904. Z. Phys. d. pflanzl. Organisation. Leipzig.]
[FALKENBERG. 1901. Die Rhodomelaceen d. Golfs v. Neapel. Berlin.]
GOEBEL. 1880. Bot. Ztg. 38.
GOEBEL. 1884. Die gegenseit. Bez. d. Pflanzenorgane. Berlin.
GOEBEL. 1893–95. Flora, 77, 38; 81, 195.
GOEBEL. 1898–1901. Organographie. Jena.
GOEBEL. 1903. Biol. Centrbl. 22, 385.
[GOEBEL. 1905. Flora, 95, 384.]

Hansen. 1881. Abhdl. d. Senkenbergschen Gesell. 12.
Hering. 1896. Jahrb. f. wiss. Botanik, 29, 132.
Hildebrand. 1898. Die Gattung *Cyclamen*. Jena.
Hofmeister. 1868. Allg. Morphologie. Leipzig.
Jost. 1891. Bot. Ztg. 49, 485.
Jost. 1893. Ibid. 51, 89.
Jost. 1895. Jahrb. f. wiss. Botanik, 27, 403.
Jost. 1899–1902. Bot. Ztg. 57, 193; 60, 21; 60, II, 225.
Klebs. 1903. Willkürliche Entwicklungsänderungen bei Pflanzen. Jena.
Leisering. 1902, a. Flora, 90, 378.
Leisering. 1902, b. Jahrb. f. wiss. Bot. 37, 421.
Leisering. 1902, c. Ber. d. bot. Gesell. 20, 613.
Lindemuth. 1901. Ber. d. bot. Gesell. 19, 515.
[Massart. 1898. Mémoires Acad. Bruxelles, 57.]
[MacCallum. 1905. Bot. Gaz. 40, 97 and 241.]
[Miehe. 1905. Ber. d. bot. Gesell. 23, 257.]
[Montemartini. 1904. Atti Ist. bot. Pavia, N.S. 10.]
Peters. 1897. B. z. K. d. Wundheilung bei *Helianthus*. Diss. Göttingen.
Schumann. 1899. Morphologische Studien. p. 238. Leipzig.
Schwendener. 1878. Mechan. Theorie d. Blattstellungen.
Schwendener. 1883, &c. Sitzungsberichte d. Berliner Akad. 1883, 1895, 1899, 1900, 1901, and Ber. d. bot. Gesell. 20, 249.
[Simon. 1904. Jahrb. f. wiss. Bot. 40, 103.]
Strasburger. 1901. Jahrb. f. wiss. Bot. 36, 493.
Vöchting. 1878. Organbildung. Bonn.
Vöchting. 1885. Jahrb. f. wiss. Bot. 16, 367.
Vöchting. 1887. Bibl. bot. Heft 4.
Vöchting. 1891. Bot. Ztg. 49, 113.
Vöchting. 1892. Ueb. Transplanation am Pflanzenkörper. Tübingen.
Vöchting. 1894. Jahrb. f. wiss. Bot. 26, 438.
Vöchting. 1899. Ibid. 34, 1.
Vöchting. 1902. Ibid. 38, 83.
De Vries. 1891. Jahrb. f. wiss. Bot. 22, 35.
Wiesner. 1889. Bot. Ztg. 47, 1.
Winkler. 1901. Jahrb. f. wiss. Bot. 36, 1.
Winkler. 1902. Ber. d. bot. Gesell. 20, 81.
Winkler. 1903. Jahrb. f. wiss. Bot. 38, 501.

LECTURE XXVII

PERIODICITY IN DEVELOPMENT. I

The developmental processes in organisms are not always carried out with the same degree of activity nor yet at an equal rate. If a plant consists of a single spherical cell this cell can increase only to a certain *specific* size; uniform and continuous surface growth with retention of the spherical form is quite impossible. In the simplest organisms growth after a certain point is followed by cell division, resulting in the formation of two organisms, each of which proceeds to develop in the same way as did the parent. Growth and division follow each other with regular periodicity. Even plants, which as a rule show no cell division (e. g. the Siphonaceae), do not grow uniformly in one direction, but from time to time form lateral branches. The more complicated the organism is the more pronounced do periodic variations in its developmental activity become, due sometimes to more or less recognizable *external* factors, sometimes to purely *internal* conditions. One of the most remarkable of these periodicities is the death after a time in many plants of part of the organism, while usually a fragment only remains alive and undergoes further development. Not less noteworthy is the phenomenon of hibernation, where all development ceases often

for weeks, months, or even longer periods, although the *capacity* for such development is still retained. These three prominent conditions—rest, activity, death—are in the highest degree characteristic of living things, and to the consideration of these and other periodic phenomena we will devote the present lecture.

This is not the first occasion on which we have encountered such problems, for we have seen elsewhere that under unfavourable external conditions, such as too high or too low temperature, or a withdrawal of water, development comes to a standstill, and finally death ensues. But plants have to endure a scarcity of water sufficient to render development impossible at regular seasons in many parts of the world, and even in our country some plants at least are periodically affected in this way. The same is true of cold, to which our native vegetation is subject every winter. Plants, we shall find, have adapted themselves in a variety of ways, all having for their object the tiding over such unfavourable conditions without suffering permanent injury. We have seen adaptations of this sort already in the behaviour of lichens and mosses to drought; such forms can remain alive despite a withdrawal of water specifically different in each case but which would at once cause the death of leaves or roots of plants higher than them in the vegetable kingdom. The seeds and spores of these higher forms, however, which, owing to internal causes, are detached from the plant, can endure the withdrawal of water and dry up, remaining alive for a long time even in this air-dry condition, or while containing a very limited amount of water. It is obvious that we cannot describe a seed in the air-dry condition as *dead*, since it may retain its *capacity for germination* for several years. The question we have to answer is whether this state of 'rest' is real and absolute or only apparent. May the seed be compared to a clock wound up and only needing a push of the pendulum to set it agoing, or is the quiescence in the dry seed not really due to abolition of vital processes but only to a diminution of these to such an extent that they cannot be recognized? The first question that occurs to us is what about *respiration*, a process which we have seen is essential to every plant activity. Does it cease in dry seeds or only greatly diminish? This problem has often been tackled, but, according to the latest critical researches, carried out by KOLKWITZ (1901), has not as yet been solved. KOLKWITZ's experiments, which were carried out on barley, show most clearly what an important bearing the amount of water present in the seed has on respiration, since 1 kg. of barley kept at summer temperature gave off in twenty-four hours 3·59 mmg. of carbon-dioxide, when 19–20 per cent. of water was present, 1·4 mmg. with 14–15 per cent., 0·35 mmg. with 10–12 per cent.

Since there is about 20 per cent. of water in freshly-gathered barley and 10–12 per cent. in air-dry seeds one must conclude that respiration decreases very rapidly as dryness is gradually attained, and in air-dry seeds reaches a value which is *practically zero*, for only about 1 per cent. of the dry weight of the seed would be respired in 100 years (compare p. 192). It is true we can accelerate the excretion of carbon-dioxide in dry seeds by raising the temperature. At 50° C. KOLKWITZ obtained 15 mg. of carbon-dioxide from a kilo of barley containing 10–12 per cent. of water. Nevertheless, we can scarcely be wrong in concluding from KOLKWITZ's experiments that respiration is not *essential* to the continuance of a vital capacity, since many seeds are not injured so far as their power of germination is concerned by enduring a *much more thorough desiccation*, in which cases respiration must be reduced to an amount which cannot be estimated, and thus can have no physiological significance. It is possible to reduce the amount of water in barley to 3, 2, or even 1 per cent., and SCHRÖDER (1886) has shown that barley containing only 2 per cent. of water germinated quite well after an interval of eleven or twelve weeks. No generalizations can be made from these results, and it is probable that fresh experiments may acquaint us with seeds whose power of germination ceases with the stoppage of respiration.

Seeds of the grass type, which can withstand thorough drying, as a rule retain their powers of germination only for a limited number of years, and such as may do so for fifty or more years must be considered as exceptional. What the loss of germinating power depends on in the long run is not known; but when we reflect that gradual alteration has been proved to take place in the proteid reserves present in dry seeds tending to reduce their solubility, we may conclude that specific protoplasmic bodies undergo as time goes on alterations calculated to render them functionless. At all events it is quite out of the question to suppose that death of the resting seed is brought about by using up of reserve substances in respiration.

But we must not assume that chemical changes in the interior of the seed, independent of respiration, alone are necessary to explain the ultimate death of the dried seed; we must also bear in mind the fact that many seeds immediately after reaching maturity are unable to germinate and only show power of development after a certain period of hibernation. Thus, according to KIENITZ (1880), the seeds of the ash, hornbeam, and of *Pinus cembra* begin to germinate the year after they ripen, and it is known in the case of other plants that individual differences occur among the seeds themselves (WINKLER, 1883); thus seeds of *Euphorbia cyparissias* germinate in the course of four to seven years. It is now definitely known that these variations depend on varying degrees of permeability of the testa for water, but we know nothing further as to why seeds which have *imbibed* water are prevented from germinating (WIESNER, 1902, p. 55); at most we may draw analogous conclusions from the behaviour of resting vegetative buds, a subject of which we shall have to speak later on. Undoubtedly internal factors play the chief part in determining the initiation and cessation of the resting period in seeds, whilst the hibernation of lichens and mosses would appear to depend entirely on external conditions.

If the seed be provided with the external and internal conditions necessary for germination, the plant resulting from its development exhibits numerous periodic phenomena, which are partly autonomous, partly dependent on its relation to the environment, e. g. *daily* and *yearly* periodicities.

Thus we may observe a *daily* periodicity in longitudinal growth, which is perfectly intelligible if we remember that plants are subjected to certain external factors which actively affect growth and which themselves vary periodically; thus we have a daily periodicity in illumination, in heat, and amount of atmospheric moisture. These factors act, however, so unequally and even antagonistically that it is impossible to calculate their combined effect beforehand. Even if we omit from consideration atmospheric moisture, an increase in which as a general rule accelerates growth, and confine our attention to light and heat, we still find that changes in these two factors may have the most varied results; the plants, in a word, may grow more rapidly either by day or by night. On a midsummer day a high temperature, approaching the maximum point, in conjunction with bright light retards growth, and increased growth in the evening may be due not merely to darkness but also to *cooling down* to somewhere near the *optimum* temperature. Conversely, in springtime the great reduction of temperature at night limits growth so much that the maximum growth occurs by *day* owing to the higher temperature, in spite of the retarding effect of light on growth.

In the experimental treatment of this question, where *two variables* are under investigation, we must naturally deal with them one at a time. Experiments in this direction have been carried out by SACHS (1872) and GODLEWSKI (1898, 1890), who studied the effect of variations in light intensity under constant temperature and moisture conditions. SACHS found that the rate of growth of the stem reached a maximum in early morning after sunrise, that it decreased hourly towards evening, and increased once more as darkness came on, often

before sunset; the increase continued until sunrise when the maximum was again reached. This growth curve is easily explained if we assume the increasingly retarding effect of light in the course of the day, and the effect of the withdrawal of light at night. This hypothesis has, however, no experimental foundation and the results obtained by SACHS have no general application. GODLEWSKI, experimenting with epicotyls of *Phaseolus*, obtained entirely different results; he found that growth was greater by day than by night and that the maximum was reached between six and eight p.m., the minimum in early morning. He further showed that the *transition* from darkness to light acts as a stimulus, in consequence of which a sudden but *temporary* decrease in growth took place. A distinction must at least be drawn between this influence of *change* in light and the influence of *constant* illumination or darkness. Corresponding stimulatory activities due to changes in temperature appear to be generally non-existent (TRUE, 1895), but we shall see in Lecture XXXIX that they do occur in special cases.

Just as we are unable at present to understand fully the alterations in growth taking place under the influence of a simple light change, so we have even greater difficulty in explaining the *after-effect* of daily periodicity which was demonstrated by SACHS and BARANETZKY (1879) as taking place in darkness under constant temperature. These investigators observed in certain cases that variations in growth, exhibited during light variations, continued with the same periodicity in darkness all day long, and there can be no doubt that a causal connexion existed between them. PFEFFER (1881) has advanced the following explanation of these after-effects. It is based on certain phenomena which we shall have to study later (nyctitropism, Lect. XXXIX), and assumes that after a single illumination not only does a simple retardation of growth take place but that this retardation is necessarily followed after a time by an acceleration; darkness operates in the same way. If, therefore, darkening sets in at a time when, owing to illumination, growth *acceleration* has already taken place, the effect of the single stimulus is added to the after-effect, and if the total effect is maintained all day long, the after-effects will become all the more established. Evidence is not forthcoming, however, to show that a double alteration in rate of growth takes place at every application of the stimulus, and, further, where it has been observed the second alteration does not make itself apparent till about twelve hours after. This must be the case, however, if, in nature, the new stimulus is to be added to the after-effect of the old.

Under these conditions it is important to note that periodic movements, especially those with daily rhythm, occur in plants which grow under quite constant conditions, in which an after-effect of any kind is out of the question. Thus BARANETZKY has observed a *daily* periodicity in beets grown in the dark under a constant temperature, resulting from internal factors only, and therefore only accidentally showing a twelve-hourly periodicity. GODLEWSKI also demonstrated in the case of beans germinated in the dark a regular daily periodicity in growth, but this does not always take place, and is entirely absent in the case of certain kinds of seeds. We thus arrive at the conclusion that a daily periodicity in longitudinal growth *cannot* be due to external factors and their after-effects only.

Among the phenomena of *yearly* periodicity the resting period, seen in trees and shrubs, and briefly referred to above, is of special interest. Selecting examples from among our native plants, we readily notice that the quiescent period occurs as a rule in the winter months, the active period in the summer, and one would naturally attribute this to the direct influence of external conditions, and more especially to the annual rise and fall of temperature. Closer examination shows, however, that this view cannot be correct, or at least that the relation between the plant and the environment is not so simple as it appears.

The winter buds of many *trees* exhibit the rudiments of an entire shoot,

which develops in the following year—all its leaves having been laid down in the autumn. The development which occurs in spring resolves itself essentially into a longitudinal growth, leaves and internodes acquiring their definite length, and this elongation may take place in the case of the oak, beech, &c., in the course of a few days (Jost, 1891; Küster, 1898). We may describe this as a spasmodic evolution, and it is evident that a certain degree of temperature is needed to give the impulse for the *commencing* of this development, but that the *cessation* of this evolution depends not on external but on internal relations. Although new buds obviously begin to form in the axils of the leaves, or terminally, by May or June, the leaves of these buds unfold only in the following year; it is impossible, however, for any one to believe that the external conditions are unsuited for their immediate development. We may readily attribute therefore the non-unfolding of these rudiments to correlation of growth. If a shoot be *deprived* of its leaves in *early summer*, in most cases a second evolution of leaves and buds takes place (Goebel, 1880), and many trees, such as the horse-chestnut, develop shoots a second time in *autumn* if they have lost their leaves owing to unfavourable circumstances such as drought. Correlation between the fully-developed leaves and the rudiments of next year's growth, prevents an immediate evolution of the latter. In this case a mere retardation of the functional activity and not complete removal of the leaves is all that is necessary to induce the correlation to make itself apparent. Hence one sees that chestnuts or maples grown in darkness (Jost, 1893) proceed to form from the terminal bud, not merely a second, but even a third shoot, and we cannot doubt that by appropriate means, e. g. by accumulation of a sufficient amount of materials, an even greater number of shoots may be induced to form. A second shoot, the so-called 'Lammas-shoot', certainly appears regularly in many trees, e. g. in young oaks, if the foliage leaves are fully active. In such a case, owing to internal relations not fully known, the correlative inhibition is removed earlier than usual. The characteristic feature, however, of this entire group of plants is that they may be made to *increase* their production of leaves but never to *keep on* doing so. Every shoot formation is followed by a period of rest, however short, and the successive shoots are separated from each other by *scale-leaves*. We have to deal with a peculiar and unmistakable *periodicity* in the plant which manifests itself not only in the periodic succession of leaf-development but also in quantitative variations in the leaves and internodes. (Qualitative variations we shall refer to later.) Very frequently one sees that the internodes in a yearly increment increase from below upwards and then decrease :—

	Length of internodes in cm.										
Aesculus	3·5	6·5	7·5	0·5							
Acer pseudoplatanus	0·5	4·5	10·5	9·5	7·0	2·5	2·5	0·0			
Fagus sylvatica	0·4	1·0	2·2	3·2	4·7	7·0	8·0	8·0	8·0	4·8	4·5

Similarly the size of the leaves varies (beech) or they may (*Acer*, *Aesculus*) decrease regularly from base to apex (compare Tammes, 1903).

Another type of shoot evolution is seen in those plants which go on developing leaf after leaf the whole summer through, and in which the cessation of leaf-development is probably induced by external factors. Thus roses grown in a greenhouse go on forming leaves until late in December. This type is naturally of less interest; it need only be noted that it is related by transitional forms with the type previously described. In *Forsythia*, for example, there are short shoots which have completed their leaf-formation in early summer, and also long shoots which develop leaves right on into autumn and finally die off at the apex without forming any terminal bud. Between these extremes there are shoots which, after a *short rest*, shoot out anew, and others which after only forming *short internodes* once more proceed to form *long internodes*. The deter-

mining factors which lead to this behaviour in individual branches are unknown, and the conclusion that it is due to a periodicity in internal causes can scarcely be avoided.

There are many observations, however, which scarcely support the view that internal factors play an essential part in this periodicity, they rather go to prove that the periodicity in the plant still stands in a definite relation to the change in the seasons of the year. If, as is clear from what has been said, this is often incorrect as regards the *commencement* of the resting period, it is so for the close of this period, for the opening of the buds takes place as a general rule in spring, when the temperature is rising. Previous to the institution of scientific experiment on the subject gardening experience had shown that the close of the resting period, in these latitudes at all events, was dependent in many plants *only* on such a rise in temperature, and that it is possible artificially to induce leaves and flowers to appear in the middle of winter if what may be termed an 'artificial premature spring' be provided. This precocious development, however, has limits; in the case of the elder it is possible to bring about an opening of the principal buds and subsequently of the flowers by raising the temperature shortly before the beginning of November; in the summer months preceding, these buds cannot be made to develop although the organs concerned have been laid down in the bud. On the other hand, the development of these buds (but without flowers) may be induced, as already noted, in early summer, immediately after the first shoot has formed, by removal of the foliage leaves. Between these two dates there is a period of rest, i.e. from July to October, when raising the temperature has no effect. This forms the *special period of rest* which depends on internal factors only but which may be easily lengthened by external factors, but is with difficulty shortened. According to ASKENASY'S (1877) researches a *complete rest*, where growth is at an absolute standstill, does not take place in the buds. In the flower-buds of the cherry a continuous but feeble *embryonic* growth goes on from summer till the next spring. *Syringa* also behaves doubtless in a similar way. We do not know why this embryonal growth at definite times, viz. from July to October, is not followed by growth in length. Certain observations which we have yet to speak of will provide us with a starting-point for further research.

JOHANNSEN'S (1900) ingenious method of shortening the resting period by etherization is especially worthy of notice. Plants which are near the beginning or completion of their resting period may be made to send out shoots by exposure for two periods of twenty-four hours each to ether vapour. It is possible that the action of the ether is that of an anaesthetic, i. e. that certain functions which tend to retard growth in the plant are inhibited by the ether. It is, however, more likely that we have not to deal with a specific action of the *ether* itself but that other poisons may produce a similar effect. What we have to think of is the stimulatory action which we have seen poisons to possess when below that degree of concentration which is fatal, an action which affects the incitation of metabolic activity and increases respiration more especially. We have every right to assume that active metabolic processes go on in the plant during the resting period. A. FISCHER'S (1890) researches, based on the valuable observations of RUSSOW (1882), have added much to our knowledge of these processes. According to them the reserve substances in trees undergo very extensive changes in the course of the winter. In autumn the parenchyma is filled with starch; in October this starch begins to undergo dissolution, fats and, in part, also glucoses taking its place. In some trees this transformation takes place in the rind *only*, the starch in the wood remaining unaltered, whilst in others (*Tilia*, *Betula*, *Pinus*) all the starch undergoes transformation, and hence no starch can be found in these trees from November to the end of February. Starch is reformed in March and shortly before the development of the young shoots it is

present in quantities almost as great as in autumn; this starch is then dissolved and employed in the construction of new shoots. The close of the special resting period coincides in a remarkable manner with the date when the amylaceous contents are at a minimum; only when all the starch is dissolved do we succeed in inducing the development of shoots, and apparently every increase in temperature at this moment induces a progressive re-formation of starch. Conversely, the dissolution of starch in autumn can be induced by lowering the temperature. Of course these transformations of reserves must not be looked on as the *causes* of periodic rest, they are to be referred to a *common* cause, possibly to an alteration in the protoplasm itself, which governs all metabolic processes.

Detailed research on our native trees would certainly conduce to the extension of our knowledge of this interesting problem in many ways, since none of the various cultivated varieties of the same species behave identically as regards their resting periods (JOHANNSEN); so much the greater then must be the differences between different species of trees. It is also well known that different organs of the same plant exhibit striking variations in their resting periods. Secondary growth in thickness, which commences usually about the same time as the unfolding of the buds, lasts considerably longer than leaf formation, at least in those trees whose buds open spasmodically, and xylem ceases forming in summer earlier than phloem (STRASBURGER, 1891). The entire process, however, always exhibits a definite periodicity such as that described, viz. activity in summer and rest in autumn and winter, the close of the period of secondary thickening certainly not being directly dependent on external conditions. The roots of trees differ greatly in this respect from shoots. Owing to the obvious difficulty of research the problems connected with root growth have been as yet little elucidated, and investigators (RESA, 1877; WIELER, 1893; BÜSGEN, 1901; HÄMMERLE, 1901) have not been successful in making a perfect comparison of the two types of organ. This much is certain, however, that in many roots growth begins in March and continues till November or December; in the middle of summer a marked decrease in growth may be frequently observed, which never amounts, however, to a complete stoppage. No experimental researches are as yet forthcoming, especially on the influence of external conditions, such as heat and moisture, but such researches are absolutely essential before we can arrive at any decision on the periodicity of growth in the root.

Many perennials may be compared with trees as regards the mode of development of the leafy shoot. A peculiarity, however, appears in our spring flowers, where apparently the resting period is transferred to the dry season of the year, the actual summer. The commencement of the new growing period generally occurs in these plants in autumn, and shows itself first in the formation of new roots. The buds also begin to develop in October and November, but do not as yet come above ground. Further development is retarded during the winter cold, and may be temporarily at a standstill. This hibernation is, however, an induced one, and on the temperature rising all these plants are easily induced to shoot in winter. In nature the formation of flowers and foliage takes place in the early spring, according to the species, from February to May. Early in the summer the leaves fall off so that in midsummer the plant is reduced to its subterranean parts only. *Colchicum* holds a special position among spring flowers because the flowers appear, as is well known, from August to October, at the same time as the new roots, while the leaves appear for the first time in the following spring. Precedence of foliage by flowers is a phenomenon of widespread occurrence among spring plants, the peculiarity in the case of *Colchicum*, however, lies essentially in this, that external factors prevent a rapid sequence of the foliage shoots. In arctic countries and on high mountains the simultaneous formation of leaves and flowers is, however, a normal occurrence (WIESNER, 1902).

More exact investigation shows us that the differences between spring plants and trees are by no means deep-seated. The summer rest of the former may be readily compared with the absence of leaf-unfolding in summer in all spasmodically-budding trees ; the foliage leaves of such plants die off much sooner than in the case of trees, but it can scarcely be doubted that embryonic growth continues during the whole summer in the subterranean organs. Nor is the early commencement of vigorous growth in the buds in autumn so very extraordinary, since, according to ASKENASY, the buds of trees do not cease developing during the winter. The characteristic feature of spring plants lies only in this, that their periods are somewhat moved forward and that their leaves are very short lived. They also exhibit a resistance at certain times to artificial forcing ; thus tulips and hyacinths cannot be made to flower before December. In all probability the formation of shoots is more readily possible in such plants just before the beginning of the resting period ; at least certain results obtained by SCHMID (1901) may be interpreted in this sense. This author has also shown that the special resting period in the case of the potato may be shortened by external causes.

The observations which have been quoted, as well as the fact that members of one and the same plant are in a state of rest *at different times*, teach us that under given external conditions only individual functions come to rest or exhibit reduced activity, and that these resting periods cannot be directly due to unfavourable external factors. This may be proved even more readily in the case of many tropical plants which show periodic growth even though external conditions be constant and favourable. We are indebted to SCHIMPER (1898) for exhaustive studies on this subject. According to his researches a large number of trees exist at Buitenzorg in Java which at longer or shorter intervals, once to six times annually, throw off all their foliage and, often after only a few days, produce fresh foliage once more. Other plants behave even more remarkably, e.g. *Amherstia nobilis*, in which the individual branches act independently of each other, so that we may find on a single tree at the *same* time branches with terminal resting-buds and shoots in all stages of development. Trees transplanted from a temperate to a tropical climate behave just like *Amherstia*, inasmuch as they lose, not periodicity itself, but its relation to seasons. The appearance of *Magnolia yulan* in December and January in the Hill station at Tjibodas near Buitenzorg is described by SCHIMPER (1898, 266) in the following terms : ' Individual branches bare of leaves but with leaf and, here and there, flower buds ; others with young leaves and full blown flowers ; others with adult leathery leaves and withered flowers ; others with leaves with autumn colouring readily deciduous.'

In other tropical climates, always warm and moist, such as the Brazilian rain forest near Para, we find examples of periodicity, e.g. *Hevea braziliensis*. According to the observations of HUBER (1898) the full-grown tree frequently produces only *one* or often two shoots in the year after the leaves have more or less completely fallen off. The *young* trees behave in an especially interesting manner. One of these formed, during the rainy period of 1896–7, five shoots, each of which was in full leaf in thirty days, and then remained quiescent for about ten days. Each shoot had at first short internodes, then longer, and then finally shorter internodes once more. It bore scale-leaves at the base, then foliage-leaves, and terminated in a bud enclosed in scales destined to be the next shoot. The five shoots opened out on Dec. 10, 1896, Jan. 20, March 12, April 25, and June 6, 1897, respectively ; the succeeding shoots were not observed exactly, but it was established that three had been formed during the remainder of 1897 and three more in the first half of 1898. Since, however, different examples are always in different phases of development, it cannot be doubted that the periodicity is due entirely to internal causes. As the plant gets older the number of shoots decreases, and, finally, as already noted, only one appears each year. This rhythmic alternation of activity and quiescence which

appears in tropical plants is an adaptation to special periods in the year, so that the resting period is associated with the cold or dry season and the active period with the warm or wet season. This periodicity, however, cannot be at once adapted to other alternations of seasons. If plants from the southern hemisphere be brought into such a climate as ours, they are able to bring their periodicity into harmony with our climate only if they become thoroughly acclimatized. In the case of our indigenous flora periodicity may be altered only gradually, since the plants maintain their accustomed rhythm with considerable tenacity. It has already been pointed out that we can induce the formation of shoots by certain stimuli, but we cannot on the other hand lengthen the resting period indefinitely without injury to the plant. Manifestly, activity is followed by a resting period in the plant; conversely, activity follows *of necessity* a period of rest, and the periodic alternation between them is maintained with greater or less constancy. [KLEBS (1903) has shown that many plants which have well marked resting periods under natural conditions may, by *artificial* means, be made to grow continuously. The chief point to note in this relation is that one prevents the occurrence of the arrests, which increase during the course of the resting period, and that already the conditions for the development of shoots are provided at the *commencement* of the *resting period*. Although we do not in the least degree question the correctness of KLEBS's very interesting observations, still we must take exception to the framing of any general conclusion upon them. The conditions existing in trees especially, which KLEBS has scarcely at all considered, compel us to hold the view that the resting period is often not to be considered as an inevitable consequence of growth. If KLEBS's view has a general application we should be able to produce a continuous leaf formation in trees which exhibit periodic vegetative activity.]

The examples we have hitherto given of daily and yearly periodicity illustrate in reality only quantitative changes in growth; there are, however, also qualitative differences, so that at different seasons *different kinds of organs* are produced. The contrast between vegetative and reproductive organs is a case in point, but even the vegetative organs themselves do not always appear in the same forms. In the highest plants, for instance, we recognize a regular succession of scale-leaves, foliage-leaves, and bracts. These qualitative changes in the productive activity of the plant stand in close relation to the quantitative types treated of above, since in this case also a yearly periodicity is very well marked. The factors which make plants produce leaves of dissimilar form are naturally internal, inquiry into which is by no means easy. Certainly, the succession of these organs is not unalterable, for we can influence the succession to a certain extent. The insight which we gain thereby teaches us nothing more than that correlations exist between the individual organs, disturbances in which induce disturbances in the normal succession. A few examples will make this clearer. The normal leafy shoot of a tree, after producing a larger or smaller number of foliage-leaves, proceeds to form scale-leaves, forming a covering under which the next year's shoot is constructed as a terminal bud. Again, similar buds are formed in the axils of the leaves, which also commence with scale-leaves. The scale-leaves have a quite different function from the foliage-leaves, and hence we find them to possess a distinct form and structure. They do not possess large surfaces exposed to light. Chlorophylliferous tissue, permeated with vascular bundles, is wanting; they are small, compact, and closely pressed together. In their first beginnings, however, they differ in no respect, as GOEBEL (1880) has shown, from foliage-leaves (Fig. 104), and they exhibit, as these do, a differentiation into leaf-base (*G*) and blade (*L*). While in the case of the foliage-leaf it is especially the blade which develops greatly, in the bud-scale it does not do so as a rule, the leaf-base developing instead. If, some time in the spring, we remove the foliage-leaves from a developing shoot, the leaf-organs, which would

in the normal course of development have formed bud-scales, develop into foliage-leaves. The lowest of these are stimulated to become foliage-leaves although they have already advanced to a greater or less extent towards the scale-leaf formation, and all intermediate conditions occur between foliage and scale-leaves. Further up the shoot quite normal foliage-leaves are developed. As a result of this experiment we may conclude that as a general rule the scale-leaves develop under the influence of the foliage-leaves, that a certain number of foliage-leaves have their activity diverted to the formation of scale-leaves. The same is true of the reproductive-leaves. The simplest form occurs in certain ferns, e. g. *Blechnum* and *Struthiopteris*. The reproductive-leaves are in this case foliage-leaves which, owing to the formation of reproductive organs (sporangia), have taken on another function and appearance. While in many ferns these sporangia arise on ordinary foliage-leaves, which retain their assimilatory function, in those mentioned above a division of labour takes place, some leaves devoting themselves only to the production of sporangia and having their assimilatory parenchyma reduced. GOEBEL has shown that if the foliage-leaves of *Struthiopteris* be cut off the reproductive-leaves which appear later (sporophylla) may change into ordinary leaves bearing no sporangia.

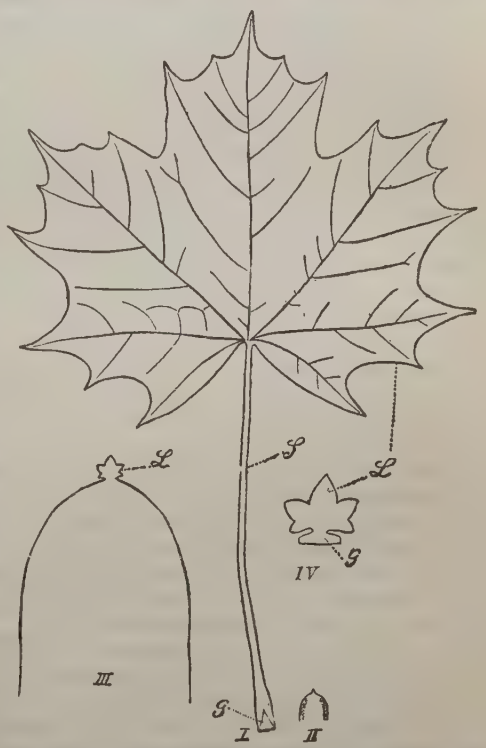


Fig. 104. *Acer platanoides*. *I*, foliage leaf, reduced. *II*, scale-leaf. *III*, young scale-leaf (magn.). *IV*, young foliage-leaf (magn. and schem.). *G*, leaf-base; *S*, stalk; *L*, blade. From GOEBEL, Organographie.

The sporophylla of the ferns are represented in the higher plant by the stamens and carpels, which are distinguished also by bearing sporangia. Further, we can determine here also tracts as forming transitions between foliage and the essential floral organs. Numerous observations and experiments have been made which show that reproductive-leaves may become altered into foliage-leaves owing to unknown causes, or, in individual cases, owing to the influence of insects, not as yet investigated (PEYRITSCH, 1882), even although it is quite obvious, both by their position and structure, that they are intended for floral organs. We must therefore conclude that in the normal plant development there are *internal* but by no means unalterable causes which induce a periodic alternation or 'metamorphosis' of leaves.

These factors are, in detail, quite unknown to us. One *hypothesis* by way of explanation has been advanced by SACHS (1880–1). According to this author there are in the leaf, in addition to the products of assimilation previously spoken of, specific constructive materials which pass away from the leaf in all directions and which collect in certain quantities where a *definite* organ has to be developed. Thus the flower would be formed out of flower-building material, roots out of root-building substance and so on.

This hypothesis very conveniently explains anomalies and regeneration phenomena, and this has won it a certain amount of acceptance. Closer examination shows, however, that the difficulties are not thereby removed, but only shifted elsewhere. If we ask what these specific constructive bodies are, why they pass to certain definite places, and how their morphogenetic activity is conditioned, we are compelled to say we have no knowledge ; hence it has been said that SACHS's hypothesis is merely a paraphrase of the facts, which moreover is not correct. A criticism of this hypothesis is to be found in VÖCHTING (1899), PFEFFER (Phys. II, 234), and KLEBS (1903) [also GOEBEL, 1905]. According to our view, the specific constructive substances for each organ cannot be transported from place to place, they consist of *protoplasm*, which remains stationary. We must assume, however, that the protoplasm of *every young* cell has the *power* of constructing the most varied type of organ. That it forms one organ rather than another is determined by a *releasing* force unknown to us and not by any primary constructive material.

Side by side with the external 'metamorphosis' in the shoot is another 'metamorphosis' in stems, branches and roots, whose seat is not the apical meristem but the intercalary meristem known as cambium. As is well known, this cambium produces different tissues inwardly and outwardly, which are termed secondary wood and secondary bast. The construction of the wood takes place in such a way that, in the course of the vegetative period, the cambium does not always produce similar elements, those formed in the beginning of spring are different from those formed in the height of summer, and these we term spring wood and autumn wood respectively (for literature see WIELER, 1891, 1892, 1897 ; JOST, 1891, 1893). Spring wood merges gradually into autumn wood but the transition from autumn wood to spring wood is more abrupt, and hence the various annual rings or single year's growths are often clearly visible to the naked eye. The difference between spring and autumn wood in the simplest cases, e.g. the Coniferae, lies merely in the decrease in the diameter of the tracheides and the increase in the thickness of their walls. This simple condition had long since suggested an explanation of annual rings. Sometimes purely mechanical factors, such as the pressure of the cortex, sometimes nutritive influences were given as the causes. No one believes any longer in any real influence of cortical pressure on annual ring formation, but it is undeniable that cells *may* develop very differently under different nutritive conditions ; so long, however, as we have no experimental data available as to the influence of nutritive substances on simple objects any explanation of secondary increase in thickness must remain imperfect.

These explanations are, besides, quite inadequate when we think of the annual rings of Dicotyledons, where, in addition to quantitative differences, qualitative differences also occur between autumn and spring wood, as, for example, in the more abundant or exclusive formation of large vessels in spring. It is naturally impossible to give a definite answer as to the effect of nutrition in such specific cases, and so no theory has been hazarded as to the special problem of annual ring formation. We must simply accept the view that this periodicity in the annual ring is due to internal factors, just as we have regarded the yearly periodicity in longitudinal growth also as autonomous. But just as in opposition to the rule there are conditions when a second shoot in the same year may be observed, or induced to form experimentally, so it is, too, with secondary growth ; a second annual ring may be seen in the horse-chestnut in autumn when supplementary shoots appear. A certain relation, in fact, subsists between the annual ring and the annual shoot. If the second shoot appears before the autumn wood has been formed, one would not expect a second annual ring (oak, Lammas-shoot) ; the relations between annual rings and annual shoots do not, however, appear to be of the nature of correlations as was previously believed (JOST, 1891), so that the formation of leaves may not

be the direct consequence of spring wood formation, both phenomena may depend on common factors, factors which operate in such a way as to induce the formation, after a certain resting period, of a new shoot with foliage-leaves and a new zone of wood with broad vessels. This concurrence is always appropriate, since with every new leaf a rise of transpiration takes place, to replace which increase in the water conduits is necessary. As to the annual rings in tropical trees so little is as yet known that it is needless for us to consider them (HOLTERMANN, 1902 ; [URSPRUNG, 1904]).

In addition to the daily and yearly periodicity in the plant a few words may be said in conclusion as to *periodicity in its entire life-cycle.* There are plants like *Stellaria media, Senecio vulgaris,* &c., which go through their entire life-history, from the germination of the seed to the ripening of new seed, in a few months, in which also each seed germinates at once, so that several generations may be formed in one calendar year without any rest and unrestricted by the time of year. After a certain number of seeds have been formed the plant dies off, but the seeds provide for the maintenance of the species. Similarly, but more intimately associated with the seasons are numerous annuals, and with these forms may be associated other monocarpic or single fruiting plants, in which the formation of seed is preceded by a two to many year stage of purely vegetable growth with or without interpolated resting periods. The probability is that in all these cases the formation of fruit is the cause of the death of the vegetative organs, since life in the latter may be markedly prolonged by preventing the setting of seed. To a certain extent in opposition to these types there are plants, such as our native trees, which fruit frequently, and whose continued existence is not determined by the formation of seed. In all perennial types another periodicity, in addition to the annual periodicity, makes its appearance, which we will study only as it is represented by trees. A tree is at the commencement a seedling with very limited power of growth, as is seen in many annuals; it gradually gains in strength, however, and by growth in length and in thickness, and by the development of the constituent elements of the xylem it attains ever increasing size until a maximum is reached. Similarly there arises also as a natural necessity a descending curve, which finally ends in death after the tree has gone on for many years producing seeds for the maintenance of the species. Long before the specimen in question as a whole succumbs, individual parts of it die off. Thus the leaves die off after they have performed their functions for one or more years, and although external factors co-operate in the leaf-fall of deciduous trees, still leaf-*fall* is as much an organic process as leaf-*formation.* As a rule, certain cells are produced at the base of the leaf-stalk whose function it is to cut the leaf off. These cells form a separating layer, the swelling up of a certain middle lamella of which brings about the separation of the dying part from that which still remains alive. As in the case of leaves, so also entire branches may be abstricted, or, without any such separation, may die and gradually rot away where they were developed. All the older tissues of the stem die in the long run ; the peripheral tissues become transformed into bark, falling off or forming a protective sheath to the parts within ; centrally the wood becomes transformed into duramen involving death of the elements. Only the apical and intercalary merismatic regions, as also their youngest derivatives, remain alive in an old tree. We thus see that every cell which has lost its embryonic characters dies after a longer or shorter period if it does not assume these characters anew, for reasons of which we have spoken in Lect. XXVI. Whether, however, a cell remains or becomes embryonic depends on its relations to the whole plant and to its different parts, for the organism provides for the persistence of some cells and the death of others. But this is not true of all organisms; where there is no differentiation into embryonic and somatic cells, as in unicellular types, there are no cells to die off of their own accord, all remain alive so long as they are not injured by accidents from without. We will refer again to this

conclusion in the next lecture. We need only add at present that the capacity for life in embryonic cells is retained only if they be active and have the power of growing and dividing; if this capacity be lost they are doomed sooner or later. We have already shown that in seeds and similar structures the inactive protoplasm for long (but not indefinitely) retains its power of development; active merismatic regions die off much more rapidly if they be prevented from growing. Root apices, for example, confined within plaster of Paris died off in about ten weeks according to PFEFFER's experiments (1893, 356).

Bibliography to Lecture XXVII.

ASKENASY. 1877. Bot. Ztg. 35, 793.
BARANETZKY. 1879. Mém. de l'acad. de St. Pétersbourg, VII, 27.
BUSGEN. 1901. Allg. Forst- u. Jagdzeitung (August).
FISCHER, A. 1890. Jahrb. f. wiss. Bot. 22, 73.
GODLEWSKI. 1889–90. Anzeiger d. Akad. in Krakau.
GOEBEL. 1880. Bot. Ztg. 38, 753.
GOEBEL. 1887. Ber. d. bot. Gesell. 5, p. LXIX.
[GOEBEL. 1905. Flora, 95.]
HÄMMERLE. 1901. Fünfstück's Beitr. z. wiss. Bot. 4, 149.
HOLTERMANN. 1902. Sitzungsber. Berliner Akad. 656–74.
HUBER. 1898. Bot. Centrbl. 76, 259.
JOHANNSEN. 1900. Das Aether-Verfahren beim Frühtreiben. Jena.
JOST. 1891–3. Bot. Ztg. 49, 485; 51, 89.
KIENITZ. 1880. Bot. Centrbl. 1, 53.
KLEBS. 1903. Willkürl. Entwicklungsänderungen. Jena.
KOLKWITZ. 1901. Ber. d. bot. Gesell. 19, 285 (comp. Blätter f. Gerstenbau, etc. Nov. 1901).
KÜSTER. 1898. Fünfstück's Beitr. z. wiss. Bot. 2, 401.
PEYRITSCH. 1882. Jahrb. f. wiss. Bot. 13, 1.
PFEFFER. 1881. Pflanzenphys. 1st ed. II.
PFEFFER. 1893. Druck u. Arbeitsleistung (Abh. K. Gesell. Leipzig, 20).
RESA. 1877. Periode der Wurzelbildung. Diss. Bonn.
RUSSOW. 1882. Sitzungsber. Dorpater Naturf. Gesell. 6, 492.
SACHS. 1872. Arbeiten bot. Inst. Würzburg, 1, 99.
SACHS. 1880–1. Arbeiten bot. Inst. Würzburg, 2, 452 and 689.
SCHIMPER. 1898. Pflanzengeographie. Jena.
SCHMID. 1901. Ber. d. bot. Gesell. 19, 76.
SCHRÖDER. 1882. Unters. bot. Inst. Tübingen, 2, 1.
STRASBURGER. 1891. Die Leitungsbahnen. Jena.
TAMMES. 1903. Verh. K. Akad. Amsterdam, 2, Sekt. IX. Nr. 5.
TRUE. 1895. Annals of Botany, 9, 390.
[URSPRUNG. 1904. Bot. Ztg. 62, 189.]
VÖCHTING. 1899. Jahrb. f. wiss. Bot. 34, 1.
WIELER. 1891. Allg. Forst- u. Jagdzeitung (March).
WIELER. 1892. Tharandter Forstl. Jahrb. 42, 72.
WIELER. 1893. Cohn's Beitr. z. Biologie, 6, 1.
WIELER. 1897. Tharandter Forstl. Jahrb. 47, 172.
WIESNER. 1902. Biologie d. Pflanzen. 2nd ed. Vienna.
WINKLER, A. 1883. Ber. d. bot. Gesell. 1, 452.

LECTURE XXVIII

PERIODICITY IN DEVELOPMENT. II

REFERENCE was made incidentally in the last lecture to the periodic alternation of foliage and reproductive-leaves in ferns and flowering plants, and the reason for the difference in form and anatomical structure of these two types of leaf, we found, lay in the different functions fulfilled by them. The reproductive-leaves have assigned to them the formation of sporangia, the specific

organs of reproduction. What is the factor which all at once determines this change in the organism, viz. the arrest of the formation of vegetative organs and initiation of the development of reproductive-leaves ? This is the problem in developmental physiology which forms the subject of consideration in the present lecture. First of all, let us be sure that we understand what 'reproduction' really means.

The current conception of reproduction has been derived from the consideration of the phenomena as exemplified by the higher plants, and especially by animals. In animals the individual is said to reproduce when it gives rise to individuals like itself. The conception of the 'individual' as understood by the zoologist cannot be applied to the plant world, for in many growth-phenomena it is extremely difficult to say whether we are dealing with continued growth of the parent or with the formation of a new organism. No one would dream of applying the term 'reproduction' to the formation of a lateral branch on the stem of a willow, for the young branch is exactly like the parent, and obviously is merely adding to the extent of the original tree ; but suppose this very branch were torn off by a gale of wind, and suppose it rooted itself and developed into a sapling, we have to decide now whether this is a reproductive phenomenon or not. The isolation of twigs, which may in this illustration be purely accidental, occurs in many plants quite definitely and regularly. Thus in many rhizomes the branches become isolated by decay of the older parts behind, and these isolated branches give rise to new and independent plants. In such cases what must one call this process ? At the beginning it is obviously merely growth and branching, later on it becomes reproduction. Where must we draw the line of demarcation ? The difficulties do not decrease when simple forms such as Algae are considered. *Spirogyra*, for instance, consists of cylindrical cells united into cell filaments. Each cell is completely independent ; it grows and divides, and thus the filament increases in size. Under definite external conditions, however (Benecke, 1898), the filaments break down into their constituent cells, each of which has the power of developing into a new filament. Now if we regard the *cell-filament* as a unity, reproduction consists in the segmentation of the thread into individual cells, but if each *cell* be an individual we have no reproductive process represented here at all.

With these examples before us we are justified in saying that often in nature no hard and fast line can be drawn between vegetative growth and reproduction. It will serve our present purpose better, however, if we institute an arbitrary limit and regard the process of reproduction as always involving the formation on the part of the plant of *special organs* whose duty it is to give origin to new individuals of the same species. Such organs must of necessity be capable of separating from the parent plant ; they must contain a certain amount of protoplasm capable of development and with possibilities of access to reserves of food-material required to render further development possible. This protoplasm may be in the form of a single cell or a cell-complex, whether occurring in higher or in lower plants, and the cell-mass may take a form as complex even as a growing point or bud. Let us commence with a simple example.

Ulothrix zonata is an alga whose cylindrical cells are combined to form a simple unbranched filament, but it differs from the majority of the forms of *Spirogyra* to which we have previously referred in being composed of cells which are not all alike ; one of them, the basal cell, exhibits the peculiarity of acting as a fixing organ (Fig. 105, *A*, *r*). Reproduction in the alga takes place as follows. The contents of a cell subdivides into two or more cells (*B*), each of which contains, in addition to protoplasm, a chloroplast and a nucleus. Further, each is provided at its colourless anterior end with four delicate protoplasmic filaments or cilia, by whose vibrations the naked cell moves through the water (*C*) after its escape from the mother-cell through a cleft in the membrane. It has been customary

to designate the reproductive organs of the lower plants as spores, so that we may describe these motile spores as 'swarmspores'. Such a swarmspore after a few hours' motility comes to rest, throws its cilia off, envelops itself in a cell-wall, and grows into a new cell-filament. Another type of swarmspore also occurs to which the name of *gamete* is given. These swarmspores are distinguished from those already described not only by the fact that they are smaller and possess only two cilia (*E*) but also especially by their subsequent behaviour. After escaping from the mother-cell (*D*) they come together in pairs and fuse with each other, forming the so-called zygotes (*F–H*). The zygote assumes a thick investment (*J*), and, after a long resting period, germinates, giving rise to four ordinary swarmspores (*K*).

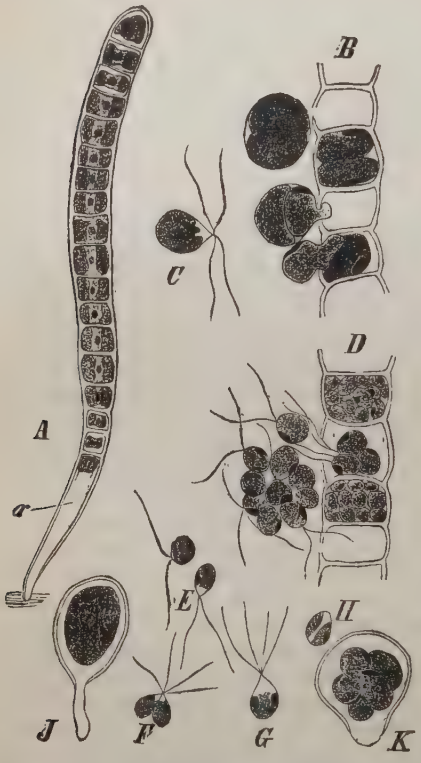


Fig. 105. *Ulothrix zonata*. *A*, young filament with rhizoidal cell, *r* (× 300). *B*, portion of a filament with escaping swarmspores, two in each cell. *C*, a swarmspore. *D*, formation of gametes and emptying of a portion of the filament. *E*, gametes. *F*, *G*, conjugation of gametes. *H*, zygote. *J*, zygote after the end of the resting period. *K*, zygote whose contents have divided into swarmspores. *B–K*, × 482. After DODEL-PORT, (from the Bonn Textbook).

Let us now compare the reproductive phenomena as seen in *Ulothrix* with those of the freshwater alga, *Oedogonium*. Here also we meet with a cellular filament fixed at one end to the substratum, also exhibiting two types of reproduction, i. e. by swarmspores and by fusion of cells. The swarmspores in this case originate, as a rule, *one* in each cell, so that the whole of the cell-contents goes to form one swarmspore; in this case no increase in number is associated with reproduction. It is of no consequence for our present purpose that the swarmspores of *Oedogonium* are structurally different from those of *Ulothrix*, all that we need lay stress on is the fact that, as in *Ulothrix*, after a certain time the movement in the swarmspore ceases and a new cell-filament is formed. So far as the other method of reproduction is concerned considerable difference exists. In *Oedogonium* the two cells which unite to form the zygote are quite different from each other, they originate in different cells, and often in different filaments. The contents of certain cells, distinguished by their greater size, and known as oogonia, contract, and the cell-wall develops a special opening (Fig. 106). The cell-contents do not escape, however, but fuse *in situ* with another cell which enters into it through this opening. These other cells have the form and power of movement of the ordinary swarmspores, but differ from them in their smaller size, reduced amount of chlorophyll, and their mode of development (Fig. 106, *III*). The large cell is known as the 'ovum', the small cell as the 'spermatozoid'. After these two cells have fused, the zygote, or, as it is here termed, the 'oospore', becomes surrounded by a thick membrane, passes through a hibernating period, and then germinates, giving rise to ordinary swarmspores. The terminology we have used, viz., ovum and spermatozoid, indicates that we have here to do with a *sexual* act comparable with that met with in the higher animals. The ovum is the female, the spermatozoid the male element, and it

is only after their fusion that further development can take place ; at the same time, however, there is one point of difference between the two cases, viz. fusion in the plant is followed by a resting period, entirely absent from the animal. It is impossible to doubt, however, that the reproductive cells in *Oedogonium* have arisen from ordinary swarmspores, and that the plant itself has been derived from forms characterized by the possession of motile gametes which fuse in pairs as is still the case in *Ulothrix*. These cells were originally alike, and a differentiation has gradually appeared in them, to which we apply the term '*sexual*'.

It was only natural that the discovery of sexuality in the lower plants (PRINGSHEIM, 1855), comparable to the only form of reproduction in the higher animals, should have created an immense sensation, and that from that time onward an attempt was always made to determine, in the first instance, in every account of reproduction whether the process was sexual or asexual (vegetative), for it was the custom to refer the extremely varied forms of organisms to one or other of these two categories. This view was, however, incorrect for two reasons. In the first place, it was shown that sexual reproduction had arisen at several points in the plant world and that possibly it had not the same physiological significance in all groups. Under vegetative reproduction, also, were included a variety of processes which could not well be homologized. It is perfectly obvious that the 'vegetative' swarmspores of *Oedogonium* have far more points in common with the ovum and spermatozoid than with a slip of a willow, and yet it is still the custom to treat of such swarmspores side by side with 'cuttings' in a chapter on 'vegetative propagation'. Unfortunately, it is not possible at present to replace this old-fashioned classification with one based on the knowledge now in our possession, although attempts have already been made in this direction. Thus HANSTEIN (1877) distinguished between reproduction by 'embryos' and by 'buds', and MÖBIUS (1897) has attempted to carry out this subdivision more fully. Without giving the reasons for the view we hold, it appears to us that this classification does not meet the case, so that we must continue to use the old nomenclature in spite of its defects. We are entitled to look forward to rapid progress in this subject as a result of the renewed interest taken lately in problems connected with the physiology of reproduction (compare KLEBS, 1900 a), although at the same time we must not expect that the infinite variety of reproductive processes in nature may ever be brought under one uniform system.

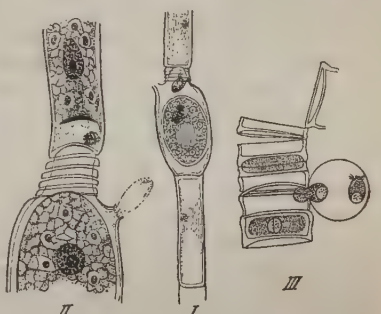


Fig. 106. *I, Oedogonium boscii.* Fertilized oogonium. The ovum is already surrounded by membrane ; above, a spermatozoid. After KLEBAHN (Jahrb. f. wiss. Bot. 24, Pl. 3). × 175. *II, Oedogonium boscii.* Young, just opened oogonium, in and in front of the opening, mucilage. After KLEBAHN (ibid.). × 400. *III, Oedogonium Vandsboroughi.* Spermatozoid formation. In the lowermost cell the spermatozoids are forming by bipartition; in the cell above it they are escaping by a fissure in the wall. After HIRN (1900).

Let us inquire now as to the factors which render reproduction in general possible, and those which are essential to the appearance of the different types of reproduction more especially in these Algae. For a long time it was imagined that these factors were essentially *internal*, and that in the lower plants, more especially Algae, just as in the higher plants, asexual and sexual reproduction alternated regularly. It was assumed that reproduction was a necessary result of internal development when the plant had reached a certain size or age. It is owing to KLEBS's (1896 onwards) researches that our insight into this question is now deeper and more exact. The chief result of KLEBS's investigations is briefly that under appropriate external conditions growth and division only take

place, but also that reproductive organs are formed only under definite conditions, and that the *nature* of these organs is not determined by internal causes alone. Internal factors, here as everywhere, play, doubtless, an important part, inasmuch as they bring about the state of activity of the plant which enables it to react to the outer world in definite ways; the environmental factor acts merely as a releasing stimulus.

Let us now examine more in detail the phenomena of reproduction as seen in *Ulothrix*. This alga grows in flowing water, in good light and at low temperatures. For these reasons it is not well adapted for laboratory research, and hence KLEBS's attempts to elucidate its reproductive physiology were not altogether successful. He was unable to induce the alga to form swarmspores or gametes at will, but he managed to determine that external factors *did* play a part in the question. Under favourable natural conditions the alga produces no reproductive organs, it simply *grows* vegetatively, and its filaments become 20 to 30 cm. in length. The principal inducement to the formation of zoospores appears to be the cessation of the flow of water and a diminution of oxygen in it. Such a condition of things takes place *periodically* in regions inhabited by *Ulothrix*, and hence for the most part growth and swarmspore formation go on at the same time. The alga may exist in this condition for years; gametes are formed only under special circumstances, apparently when the water level falls and when the cells come directly in contact with air, or are only occasionally sprinkled with water. The zygote can withstand drought, and hence it is intelligible that they should be formed under dry conditions.

KLEBS (1896) obtained more satisfactory results with species of *Oedogonium*, which are more easily cultivated than *Ulothrix*. The two species investigated behaved differently, but one can only say that both demanded a certain alteration in the surroundings before swarmspore formation could be guaranteed. In the case of *Oedogonium capillare* darkening had a quite marked effect, which was increased by cultivation in a 4 to 10 per cent. solution of cane sugar. After being cultivated in ordinary water, in which it assimilates vigorously, it produced swarmspores on being transferred to a dilute nutrient salt solution. In *Oe. diplandrum*, on the other hand, swarmspores were formed when it was transferred from flowing to still water, from a lower to a higher temperature, from an inorganic nutrient solution to pure water. This is all the more remarkable seeing that both forms grow in similar localities. It is very desirable that these two plants should be more closely studied under natural conditions, so that we may get to know under what circumstances the formation of swarmspores takes place in nature; at present we are acquainted with the artificial conditions only. Sexual organs may be produced in both species if the water be limited in amount, and if it contains little in the way of nutrient salts, and if the plants be strongly illuminated. *Oedogonium diplandrum* requires a much greater intensity of light than *Oe. capillare*.

Sexual reproduction is by no means essential to the persistence of *Oedogonium*, for KLEBS has shown that *Oe. diplandrum* grows luxuriantly in many situations without ever forming sexual organs, and that only the male form of *Oe. capillare* occurs near Basel, thus entirely precluding the formation of zygotes. The biological distinction between zygotes and swarmspores is perfectly obvious. By means of the latter the plant is able to reach new locations and by the former to tide over periods when external conditions are unfavourable to vegetative growth. Put shortly, swarmspores subserve distribution, zygotes, continuity of the species. Why a *fusion of two* cells is necessary for the formation of a resting spore and why these two cells should exhibit sexual differences is not so apparent. On reviewing the reproductive processes in Algae and Fungi we find that although very often sexual fusion is followed by the formation of resting spores, still there are cases of resting spores produced asexually (e. g. Bacteria), and, on the other hand, products of sexual union which develop

directly without any resting period (e. g. *Fucus*). If follows that sexual fusion is not the cause of the need for a resting period ; indeed it has been observed that the gametes of *Ulothrix* under certain circumstances (KLEBS, 1896, 321) may develop into resting spores *without any fusion*, and the same phenomenon has been for a long time known to occur in many species of *Oedogonium* (compare HIRN, 1900, p. 39). If gametes are able to develop into resting spores without any fusion, then conjugation must have some special significance of its own, into which we shall go later on.

The examples quoted are sufficient to render clear the historical lines of investigation on this subject. Fungi are, however, much better adapted for studies of this kind than Algae, because we are more thoroughly acquainted with their conditions of life, and because these conditions are for the most part not so restricted as in the case of Algae. An example of this has been already given in *Basidiobolus ranarum*, but without referring further to this case we may rather turn to the *general conclusions* which KLEBS (1900a) has arrived at from his researches on Fungi. In that group, far better than in Algae, we see that reproduction is not an essential result of vigorous growth, as one would be inclined to assume from the regularity with which reproduction follows such growth. As a matter of fact, growth may proceed to an unlimited extent without reproduction, provided the necessary external conditions are fulfilled. In addition to favourable general conditions the most important is continuously good nutrition, e. g. in cultures, a frequent renewal of the nutrient solution before its nutritive value has appreciably diminished or injurious metabolic products have accumulated. If, from time to time, a fragment of the mycelium of *Saprolegnia mixta* be taken from a good culture of the fungus and transferred to a fresh nutritive solution, it may be kept growing vegetatively for two and a half years without reproducing. 'This phenomenon is illustrated far more effectively by the case of yeast, which has, for hundreds of years, persisted in the vegetative condition, and which can be induced to form spores only under very special conditions.' Similar experiments have been carried out on a large number of lower organisms, Myxomycetes, Bacteria, and Fungi, with the same results ; it is more important to note that at least *some* higher types may be similarly treated, e. g. *Coprinus ephemerus*, which may be cultivated for months as a sterile mycelium without the formation of any pileus. Experiments cannot be carried out so successfully on Algae because it is often the case that the most *insignificant alterations* in the conditions induce spore formation ; it may be noted, however, that *Vaucheria geminata* will remain sterile in flowing water for as long a period as may be desired. The factor which most commonly induces the appearance of reproductive organs is an *alteration in nutrition*, especially a *reduction in the absorption of food*. In the case of certain Fungi which form fruits only in the air, *transpiration* is also a determining factor ; a saturated atmosphere interrupts the formation of spores, but a reduction in the amount of moisture induces the formation of spores in abundance. *Light* also plays a part in a few cases, as in certain species of *Coprinus* and in *Pilobolus microsporus*. It is only when light is permitted to play on the terminations of the conidiophores of *Pilobolus* that conidia are formed. Illumination for a few minutes is often sufficient; if it be not illuminated the conidiophores grow on just like ordinary vegetative cells, so long as nutritive materials can reach them.

The withdrawal of nourishment acts in the first instance as a stimulus to the formation of reproductive organs, while, at the same time, it retards vegetative growth. A contrast would thus appear to exist between reproduction and growth pure and simple, but it must not be assumed that reproduction begins when nutritive conditions are unfavourable and growth only when they are favourable. On the contrary, it appears that reproduction is all the more vigorous the better the vegetative parts are nourished. It would thus appear that growth is an *essential precedent* of reproduction. It is so, however, only in

so far as growth is associated with vigorous assimilation, for it is obvious that the construction of reproductive organs necessitates the previous accumulation of a certain amount of nutritive materials, and these must be all the more abundant the more complex the reproductive organs are. Thus simple reproductive bodies may arise from other organs of propagation *without growth*, provided these have a sufficient supply of constructive materials. The best known example of this is seen in the swarmspores of *Oedogonium* which after coming to rest may become swarmspores once more. KLEBS has drawn attention to similar phenomena in many Fungi.

A more important difference between growth and reproduction lies in this, that general vital conditions are not so *restricted* in relation to the former as the latter. For example, growth may still go on at a very low or a very high temperature, while formation of reproductive organs ceases. Oxygen, light, concentration of the nutritive solution, the quality of the nutrient, all have minima and maxima which are much closer together in the case of reproduction than in the case of growth. It must not be assumed, however, that the optimum for the one process is also that for the other.

Observations similar to those which have been made on Algae have also been carried out on Fungi, both as regards the occurrence of the reproductive organs in general and of the various types of these in particular. Fungi far excel Algae in the variety of their methods of spore formation. Interesting as are the results achieved by KLEBS and his pupils on this subject we must not attempt to describe them here, for their complex nature renders it impossible to summarize them in a few sentences, and space will not permit of more.

If we turn now to the *higher* plants, at first sight we appear to meet with an essentially different state of affairs. An oak, for example, has, like a higher animal, only *one* type of reproduction, i. e. the formation of an embryo contained in a seed. This embryo is the product of a sexual act, which takes place when the plant has reached a certain age, and is seemingly induced by *internal* causes. Closer study, however, reveals something entirely different. In order to understand the phenomena we must study reproduction in the fern, for a knowledge of the process in the latter is essential to a true conception of what follows. The fern has the same complicated structure as the flowering plant, consisting as it does of a root-system and a leafy shoot. On the under sides of the leaves arise, in definite ways characteristic of the group, asexual spores enclosed in special receptacles, the sporangia. The spores generally pass through a resting period and germinate under favourable conditions. The plant which arises from the spore resembles, however, a liverwort rather than a fern. It consists of a *cellular expansion* a few millimetres in diameter, increasing by means of an apical cell, and attached to the soil by rhizoids. This second generation derived from the spore is termed the prothallus, and is incapable, as a rule, of developing by simple growth into the first stage. When its reproductive period comes on it develops sexual organs. Archegonia, corresponding to the oogonia of *Oedogonium*, appear (Fig. 108), and each of these contains, as its essential constituent, an ovum. The ovum is fertilized by a motile sperm, produced in a special receptacle, an antheridium (Fig. 107), and from the product of the fusion of these two cells there arises a new fern plant, which is at first dependent on the prothallus, but later on, after the death of the prothallus, is capable of independent existence. In the natural course of events the two generations in the fern follow each other regularly, the asexual generation or sporophyte being succeeded by the sexual gametophyte, and that in turn by the sporophyte ; in other words, we have here a case of *alternation of generations*. Any attempts to induce a spore to form a sporophyte or an oosperm to form a gametophyte by external agencies have been as yet entirely unsuccessful. In this respect the fern shows a marked difference from the Algae and Fungi.

This distinction cannot, however, be fundamental, for we know of several

abnormal phenomena which occur in many ferns under conditions which have not as yet been fully studied, viz. the phenomena termed *apospory* and *apogamy* (for literature see GOEBEL, 1898–1901, p. 430). By *apogamy* is meant the development of the sporophyte, or part of it, the sporangium, as a *vegetative* outgrowth of the prothallus, without any intervention of the fertilized ovum. Again when prothalli arise not from spores but from sterile sporangial-cells, or from general vegetative-cells of the sporophyte, we speak of the phenomenon as *apospory*. Apogamy and apospory show that the characters of the sporophyte, as also of the gametophyte, lie latent in every cell of the fern, and that the changes leading to the formation of the other generation do not occur first in the egg-cell or in the spore. Apospory and apogamy lead us to hope that it may yet be possible to discover the more immediate conditions that determine the rhythm of alternation so that we may then be able possibly to alter it at will.

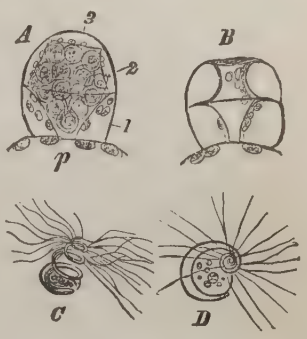


Fig. 107. *Polypodium vulgare*. *A*, mature, *B*, empty antheridium; *p*, cell of the prothallus; *1* and *2* parietal cells; *3*, cover-cells (× 240). *C*, a motile sperm. *D*, the same fixed by iodine solution (× 540). From the Bonn Textbook.

Another variation from the regular alternation of generation occurs much more frequently than apospory and apogamy. Many ferns have in addition to spores and sexual cells other reproductive organs which may be termed *accessory*. These may occur on the sporophyte or on the prothallus, but in neither case do they form stages in the *alternation*; one variety renews the sporophyte, the other the gametophyte (BOWER, 1887). The regularity in the alternation of generations may in this way be destroyed and each generation may go on repeating itself directly an indefinite number of times. Doubtless careful investigation may show that external factors often determine whether the typical or the accessory reproductive organs shall be produced in any special case.

If we now glance at the life-cycle of the other Pteridophyta and compare them with that of the ferns in the narrower sense of the term, we find that we are able to identify in all of them the same two generations, but not always with equal clearness. The gametophyte in very many forms reaches only a very limited size, and is finally entirely enclosed within the spore. The wall of the spore opens at one end, in the one case to allow the sperms to escape, in the other to afford a means of entry for the sperm to the archegonium and to the ovum. Concomitantly with these reductions in size of the prothallus, further modifications occur in its historical development. While both types of sexual organs are or may be borne on the prothalli of ferns, in other types the prothalli are unisexual, and it depends on the character of the spore and the sporangium whether the prothallus

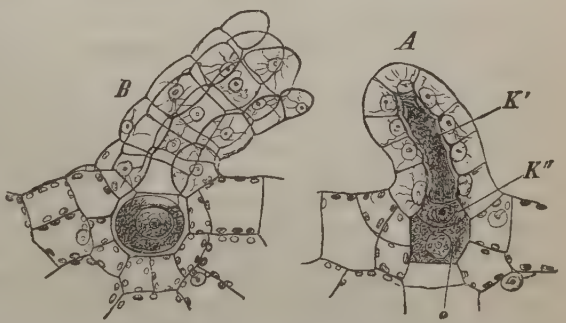


Fig. 108. *Polypodium vulgare*. *A*, immature archegonium; *K'*, neck canal-cells; *K''*, ventral canal-cells; *o*, ovum. *B*, mature and open archegonium (× 240). From the Bonn Textbook.

will afterwards produce male or female reproductive organs; the sporophyte produces megaspores in megasporangia and microspores in microsporangia, the former female, the latter male.

This stage in the historical development of the Pteridophyta brings us nearer to the condition found in the Phanerogams. As a result of HOFMEISTER'S epoch-making investigations it is possible to compare the Gymnosperms in all essential points with the Pteridophyta. We must, however, refer to the text-books on botany or to the expositions of the subject given by GOEBEL in his Organography of Plants for details of the comparison, since the plan of the present course of lectures does not include a treatment of morphological relationships such as these. We shall confine ourselves to a discussion of the Angiosperms, whose likeness to the Pteridophyta is not so apparent. Angiosperms also develop sporangia on leaves which, like those of Pteridophyta, undergo metamorphosis. These metamorphosed leaves and the sporangia which they produce are collectively spoken of as the flower. The metamorphosis of floral-leaves has been already referred to at p. 349, and it would appear that phylogenetically this metamorphosis has been intimately associated with the formation of sporangia, but whether the same is true ontogenetically in each individual case is more than doubtful. Floral-leaves, at all events, have not lost their power of becoming foliage-leaves, and after the application of appropriate stimuli flowers may be induced to take on a green colour.

This question, however, need not be considered here, for it is the *sporangium* and not the leaf from which it arises that is more especially before us. We may distinguish microsporangia (pollen-sacs) developed on staminal leaves and containing microspores (pollen-grains), and megasporangia (ovules) enclosed within carpels, each containing, for the most part, one megaspore or embryo-sac. Very frequently we find micro- and mega-sporangia united in the same flower, and their mode of occurrence is determined exclusively by internal factors, acting in such a way that the microsporangia are developed *first*, the megasporangia later.

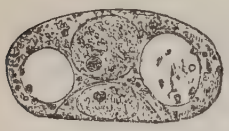


Fig. 109. *Tradescantia virginica*. Grain of pollen. Below, the antheridial, above, the vegetative-cell. × 540. From the Bonn Textbook.

The case where the flowers are unisexual, and where the sexes are distributed on different plants, requires further investigation as to the factors which bring about this determination of sex, but no definite conclusions on the matter have as yet been reached (STRASBURGER, 1900).

Let us now trace the further development of the spores. The microspore, without any further growth, becomes transformed into a prothallus, dividing into two cells of unequal size (Fig. 109). The small cell becomes the antheridium, dividing later into two male cells, the large cell remains sterile and has a special function to perform. This function becomes evident so soon as the microspore reaches the stigma through the agency of wind currents or insects. So soon as it has become securely fixed in that situation it proceeds to develop a long hypha-like pollen-tube, which (Fig. 110) forces its way deep into the megasporangium, breaks through the wall of the megaspore, and sheds into it the two sperms. The earlier changes which take place in the megaspore are very remarkable. The megaspore remains imbedded in the tissue of the sporangium, hence the necessity for the formation of a pollen-tube. Its nucleus undergoes three successive divisions, so that finally eight nuclei are present in the megaspore, and these are arranged in a definite manner (Fig. 110). Three of them are situated at the end of the megaspore where the pollen-tube enters, forming there what is termed the egg-apparatus (*ie*, Fig. 110; compare Fig. 111), each becoming surrounded by a layer of protoplasm obtained from the mother-cell. The egg-apparatus thus consists of three naked cells. Similarly, at the other end of the megaspore, three other cells (*an*, Fig. 110) are formed, the

antipodal cells, which, however, appear to have no special function to perform. Finally, there remain over two other nuclei—the polar nuclei—which move towards the centre of the megaspore, fusing sooner or later in that situation, the product being known as the secondary nucleus of the embryo-sac. Which of these nuclei or cells are to be considered as belonging to the prothallus it is impossible to say; this much, however, is certain, that the three cells of the egg-apparatus, or, at all events, the one distinguishable by its size as the ovum, represents the archegonium reduced to its simplest condition.

After entry *one* of the sperm nuclei fuses with the ovum, and the product develops into the embryo. The two cells adjacent to the ovum disappear. The other nucleus fuses with the embryo-sac-nucleus (see p. 370), and from the pro-

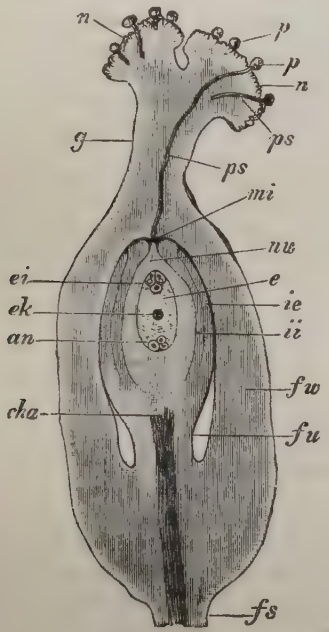


Fig. 110. Pistil of *Polygonum convolvulus*. *fu*, the funicle of the ovule, supporting the body of the ovule with its integuments, *ie* and *ii*, and containing the nucellus, *nu*. The latter encloses the embryo-sac, *e*, in which is seen the egg-apparatus, *ei*, the embryo-sac nucleus, *ek*, the antipodal cells, *an*. The ovule is enclosed within the ovary, *fw*, which is continued into the style, *g*, and ends in the stigma, *n*. On the stigma are seen pollen-grains, *p*, one of which is giving rise to a pollen-tube, *ps*. × 48. From the Bonn Textbook.

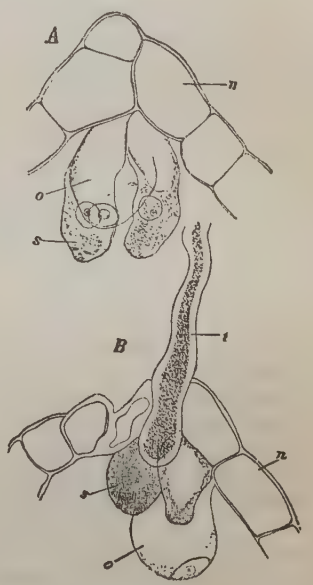


Fig. 111. *Funkia ovata*. Upper end of the embryo-sac, showing the egg-apparatus. *A*, before fertilization; *B*, during fertilization. *o*, ovum; *s*, synergidae; *t*, pollen-tube; *n*, nucellus. × 390. From the Bonn Textbook.

duct of that fusion and from the protoplasm of the embryo-sac there arises a cellular tissue—the endosperm—which becomes filled with reserve food materials, and sooner or later is used up by the developing embryo. The whole megasporangium now increases greatly in size and forms the seed, which when mature enters on a period of rest.

Even in the very highest plants it is thus possible to demonstrate an alternation of generations. The marked reduction in the gametophyte of the fern is carried in this case so far that practically nothing is left of that stage except the reproductive organs themselves, and the alternation becomes in consequence completely hidden. It is obvious from a consideration of these facts that there will be far less opportunity for variations in the regularity of the succession of generations in the higher plants than in the ferns. Since the

development of sexual organs appears to be a necessary consequent of the formation of spores physiological investigation must be limited to the interpretation of the conditions of *spore-* and *flower*-production.

As in certain ferns, so also in many Angiosperms, we meet with *accessory* organs of reproduction, at least in the sporophyte stage, and when we speak of these as asexual or vegetative reproductive methods we do not mean the mega- and micro-spores (although these also are asexual in character), but those special accessory organs which invariably consist of a growing point or bud, giving rise to leaves and roots after these or the organs bearing them have separated from the parent. Great variations occur in the point of origin of these buds, and in the way in which the necessary reserves are stored in leaf, stem, and root; these, however, we need not discuss here.

After this superficial sketch of the morphology of the reproductive organs of the flowering plant we may pass to the problems requiring consideration from a physiological standpoint, viz. what determines flower formation, what vegetative reproduction; what relations exist between reproduction and vegetative growth; what is the special *significance* of reproduction, and more especially of asexual and sexual reproduction.

We are not nearly so well acquainted with these subjects in connexion with higher plants as in the case of the lower, and the experimental investigation of the former is much more difficult. The first question we have to answer here is whether or not unlimited growth is possible *without* reproduction. At the first glance the answer would appear to be in the negative, but further consideration shows that that answer is incorrect. A tree may be several hundred years old and still go on growing; in the long run it dies, but we cannot say that death was due to *internal* factors and that perpetual vegetative growth was *impossible*. Death may be due rather to external factors, e. g. the ever-increasing difficulty of conducting water to the lofty growing points. Klebs (1903) attempts to account for the death of trees by assuming that certain decomposition products migrate from the dead parts and gradually infect the living organs. Our suggestion above might be made more general by saying that when a certain size is exceeded nutritive disturbances (not merely interruptions in the water flow) generally ensue, which in the long run bring about death. In many of the lower organisms also, e. g. in *Hydrodictyon*, continuous vegetative growth appears to be impossible. When the cells of this alga come to exceed a certain size its central cells are badly supplied with food-material and must in the long run die off unless the size of the body be reduced as a result of reproduction. *Internal* factors are also known to be effective, but they do not affect the parts of the tree which are of special interest to us. The protoplasm of the growing points in an old tree has just as much power of development as that of one a single year old. In proof of this view we point to the numberless cultivated plants which are propagated by cuttings, and which have been so propagated for hundreds of years. This is true, for example, of the willow, poplar, sugar-cane, and many others; at the same time, some of these plants in their later years are often subject to diseases which have been considered to be a consequence of degeneration due to perpetual vegetative propagation. It has, indeed, been maintained that the life of cuttings is limited by that of the mother-plant from which they are taken. Möbius (1897) has, as it appears to us, demonstrated beyond criticism that this view is incorrect, and we may refer directly to the evidence he has advanced. It will be as well, however, if we cite one or two examples in illustration of the position we hold, viz. that vegetative growth may be unlimited. At the commencement of the present lecture we referred to the case of rhizomes which grew on from year to year, in the main axis or in lateral shoots. Since they are always forming new roots acropetally all difficulties in absorption of water are removed. The older parts, however, die off, and hence it is not possible in nature to deter-

mine whether such a plant has been in existence for years or for centuries. Many of these rhizomic plants have lost their power of forming seed, and their continued existence depends entirely on the growth of the rhizome. *Acorus calamus* is a case in point. This plant has been known as a native of Europe for at least 400 years, and there can be no doubt that during the whole of that time it maintained itself in existence and increased by vegetative methods only. [KLEBS has given some admirable examples (1903–4) of continued vegetative growth experimentally induced. He was successful in causing *Glechoma hederacea* to remain growing in the form of creeping shoots for years.]

The examples quoted make it clear that the formation of flowers and reproduction by means of sporangia is not generally necessary for the tribal continuance of the plant, and that the plant, whether it be a flowering plant or a *Saprolegnia*, can continue in existence by vegetative means only. Inquiry into the factors which bring about flower formation becomes thus all the more necessary.

Fig. 112. *Mimulus tilingii*, after VÖCHTING (1893, Pl. 11). *I*, Top of a normal plant. *II*, Top of a plant cultivated in diminished light after development of inflorescence. The inflorescences (*a*, *a*) are stunted; vegetative shoots have developed everywhere abundantly.

When we examine flowering plants under natural conditions we find that the flowers begin to appear when the plant is 'ripe for flowering' just as sexual-cells appear in the animal when it reaches a certain age. But although flower formation, generally speaking, takes place at a certain *age*, which differs with each species, still, exceptions are known, as, for example, the oak, which normally is 'ripe' in its sixtieth to eightieth year, but which occasionally produces flowers in its first year and then dies down (WIESNER, 1902, p. 75. Compare Fig. 93). VÖCHTING (1893) has demonstrated in a very striking manner that flower formation is influenced by external factors. The plant he experimented upon was *Mimulus tilingii*, which left off forming flowers in light of low intensity but still sufficiently strong to permit of vigorous vegetative growth. The effect of the diminution in light makes itself felt even when the inflorescence has been formed; it arrests the further development of the flower already laid down in the axils of the bracts (Fig. 112), and induces a general evolution of resting axillary buds into vegetative shoots.

This illustration of the relationship between foliage and flower formation recalls vividly that between growth and reproduction in Fungi. We have established in relation to these forms that the limits of the general vital conditions for reproduction are more restricted, and VÖCHTING has shown in *Mimulus* that the minimum of illumination for flower formation lies higher than for that of vegetative organs. We are, however, as yet far from having reached a solu-

tion of the problem, since the increase in the intensity of light obviously does not play the same part in VÖCHTING's experiments as does the limiting of the food supply in *Saprolegnia*. A certain light intensity is only one of the conditions of flower development and not the chief releasing stimulus. Under such a light intensity of course vegetative developments go on unchecked. There are many observations and experiments which show that other external factors may be of great importance in the determination of flower formation. Ivy blooms only in a warm sunny aspect, and exhibits vegetative growth only in woodland shade (WIESNER, 1902, p. 75). The quality of the light also has an important effect on flower formation. According to SACHS (1887), ultra-violet light must be especially considered in this relation (p. 311). In his experiments, carried out under conditions when ultra-violet light was excluded, flower-buds were formed, but they did not open ; apparently the intensity of the light was too low. SACHS's statements may be explained by VÖCHTING's experiments and observations (1893) and by those of KLEBS (1900 b) and MONTEMARTINI (1903). MÖBIUS found (1897) certain Gramineae and species of *Borago* flowered better on dry soil in places where nutrients were restricted in quantity than where there were abundant supplies of water and nutritive salts. Perhaps with this is related the fact that root-pruning of trees tends to induce an increase in flower formation ; that ringing has also the same effect is probably due in the first instance to the abundant supply of organic material to the axillary buds. [KLEBS (1903, and especially 1904, 545) has established the external conditions necessary for flower formation in a number of plants. No general laws on the subject have as yet, however, been formulated ; on the contrary, every plant seems to maintain relationships of its own with the environment.]

Although external factors are known to be of great importance in flower formation, it is not to be expected that they play as vital a part there as in the lower plants. Even though proof be advanced that flower formation stands in definite relation to a definite external factor, that is not to say that that relation is so simple and so direct as in the case of a unicellular alga. If it be proved, for instance, that a dry soil increases flowering, and that a wet one retards it, we can only say that the character of the soil *directly* influences the roots and only *indirectly* the aerial organs. The root may be looked upon as a part of the environment of the shoot, just as every cell is a part of the environment of another cell, but that does not affect the relations existing between different parts of the higher plant, and calculated to affect the formation of flowers also. In fact, the correlations between leaf and flower formation must always be kept prominently in mind. All factors which tend to advance foliage development are unfavourable to flower production and vice versa. In extreme cases flowers are completely suppressed, and certain plants exhibit vegetative growth only ; in certain aquatics GOEBEL (1893) showed that the luxuriant formation of vegetative organs was the cause of the absence of flowers, and MÖBIUS (1897, p. 137) has brought forward evidence of a similar nature. We need not enter more fully into these correlations since we cannot bring forward anything substantially new in their explanation. It is always important, however, to remember that in flower formation not merely *external* but also *internal* stimuli may play an important part, and some of SACHS's (1892) observations on this question are very suggestive. In May SACHS made cuttings of *Begonia* in the usual way, and found that the plants springing from such leaf-cuttings gave rise to flowers in the beginning of November, preceded by a luxuriant formation of foliage-leaves. If, however, the leaf-cuttings are taken from a flowering specimen in the end of July, flowers appear on them in the end of September, but few leaves are previously formed. Several other experiments of the same nature are recorded. Thus GOEBEL (1901) found that leaves of *Achimenes haageana* in the flowering condition, used as cuttings, proceeded to form flowering shoots at once, while

cuttings from younger plants produced vegetative shoots. Such facts as these SACHS attempted to explain by his hypothesis of specific constructive materials (p. 349). He assumed that special flower-forming materials occurred in the plant about to flower, and hence the rapid formation of flowers on cuttings taken from it. Quite apart from the general criticisms we have brought against SACHS's hypothesis, there are by no means unimportant objections to this special application of it. WINKLER (1903) found the shoots which arose from the leaf cuttings of *Torenia* very liable to form flowers, and this tendency was held in common by *all* leaves, and even by cotyledons. On the assumption that the materials for the manufacture of flowers postulated by SACHS must be especially abundant in the *flowers themselves* I prepared flower-cuttings of many species of *Achimenes*; though these produced buds in quantities none of them formed flowers. The conditions necessary for the production of flowers from leaf-cuttings must obviously be studied on broader lines; still these facts may be adduced as interesting evidence that internal factors play a very important part in the formation of flowers.

In general, however, our information as to the factors which are essential to the formation of flowers is very imperfect; the facts which have been brought forward prove, however, that the periodicity in blooming usually observed is not fixed once and for all but may be experimentally influenced. On the other hand, the alternation of generations in the Phanerogams is in so far fixed that the formation of spores is followed necessarily by the formation of sexual organs.

Plants which, in addition to seeds, also form accessory organs of reproduction, such as tubers, bulbs, &c., require special mention, since it can hardly be doubted that the conditions which are necessary for the formation of flowers or seeds must differ from those which govern the formation of propagative buds. On this point, however, little is known; so far only *correlations* between them have been established. For example, it not infrequently happens that flowers and propagative-buds are produced concurrently, but that the setting of seed takes place only if the formation of propagative-buds is prevented (compare LINDEMUTH, 1896). The cases where propagative-buds arise in place of flowers are of especial interest, e.g. *Poa bulbosa* and many others, for in these cases we do know that special but as yet unknown external factors accelerate or retard the formation of such flowers or buds (HUNGER, 1887). [Looking at the general results we have arrived at in this lecture we must admit that the *normal* path of development, both in higher and in lower plants, is only a special type of many developmental possibilities. That this normal sequence and not some other is usually met with must be attributed to a normal sequence in the external factors. It is to KLEBS especially that we owe this valuable conception with regard to the factors governing developmental processes.]

A few words may be said in conclusion as to the biological significance of reproduction. In the lower organisms this is especially obvious, for the importance to the organism of swarmspores and of conidia and resting spores capable of distributing the plant in the air requires no explanation. The value of the formation of fruit and seed in the higher plants is equally intelligible. It is immaterial whether the organism forms these organs once and then dies, or whether it continues forming them for several years, their production tends not only to the persistence but also to the increase of the species. Every organism does its best to distribute itself and to seek pastures new; this characteristic is so marked in living things that one gets the impression that their entire existence is spent in producing their successors. We use the term 'impression' purposely, because attempts to answer such questions tend to teleology; they lead us at once to the ultimate problem of all, viz. what is the final aim and object of the existence of the organism? and to that question no answer can be given.

Although we may understand in general the biological reasons for the

occurrence of reproductive organs we must admit that we have as yet arrived at no clear appreciation of the factors which determine the existence of *several types* of these organs. There are many organisms which persist and multiply exclusively by one mode of propagation. As above observed, MÖBIUS recognizes buds and germs, and distinguishes them by their mode of origin. Germs arise by *rejuvenescence*, buds by growth and ordinary cell division. Rejuvenescence is illustrated in the formation of the ovum, as also in the development of swarmspores. It is not quite so obvious, however, why the origin of a new membrane—a phenomenon which did not occur in *Oedogonium*—should be of so much importance to the organism, since the old membrane was just as capable of growth as the new one in this case. But in describing the phenomenon as 'rejuvenescence' we are regarding not so much the cell-wall as the internal rejuvenescence of the protoplasm. STRASBURGER (1880) has shown that the protoplasm undergoes a reconstruction when swarmspores are formed in *Oedogonium*; the nucleus moves through the green protoplasm towards the outside, and later on resumes its old position. BERTHOLD (1886, p. 289) has observed similar rearrangements in other cases of cell-formation. It is not clear, however, how such processes bring about a rejuvenescence, and one idea underlies all conceptions of this nature, viz. that protoplasm degenerates, gets worn out by perpetual growth and cell division, and this conception is one which we have striven to oppose. If protoplasm wears itself out, it has also no doubt the power of regenerating itself.

There is one point which we have left undiscussed in our consideration of these questions, viz. why must the formation of many resting spores in the Thallophyta be preceded by a fusion of cells, a phenomenon which may in the extreme case be regarded as a sexual act? Why should the ovum in the Pteridophyta and the Phanerogams develop only after being fertilized by the spermatozoid? We shall consider this question in the next lecture.

Bibliography to Lecture XXVIII.

BENECKE. 1898. Jahrb. f. wiss. Bot. 32, 453.
BERTHOLD. 1896. Protoplasmamechanik. Leipzig.
[BOWER. 1887. Trans. Linn. Soc.]
GOEBEL. 1893. Biolog. Schild. 2, 217. Marburg.
GOEBEL. 1898–1901. Organographie. Jena.
HANSTEIN. 1877. Botan. Abhandl. 3, part 3.
HIRN. 1900. Monographie d. Oedogoniaceen. Acta Soc. Sc. Fennicae, 27. Helsingfors.
HUNGER. 1887. Ueber einige vivipare Pflanzen u. d. Erscheinung d. Apogamie. Diss., Rostock.
KLEBS. 1896. Ueber d. Fortpflanzungsphysiologie d. nied. Organismen. Jena.
KLEBS. 1898. Jahrb. f. wiss. Bot. 32, 1.
KLEBS. 1899. Ibid. 33, 513.
KLEBS. 1900 a. Ibid. 35, 80.
KLEBS. 1900 b. Ber. d. bot. Gesell. 18, 201.
KLEBS. 1903. Willkürliche Entwicklungsänderungen. Jena.
[KLEBS. 1904. Biol. Centrbl. 24, 257–614. Comp. also 1905, Jahrb. f. wiss. Bot. 42, 155.]
LINDEMUTH. 1896. Ber. d. bot. Gesell. 14, 244.
MÖBIUS. 1897. Beitr. z. Lehre v. d. Fortpflanzung d. Gewächse. Jena.
MONTEMARTINI. 1903. Atti Istit. Bot. Pavia. N. S. 9, 1.
PRINGSHEIM. 1855. Monatsber. d. Akad. Berlin.
SACHS. 1887. Arb. bot. Instit. Würzburg, 3, 372.
SACHS. 1892. Flora, 75, 1.
STRASBURGER. 1880. Zellbildung u. Zellteilung. 3rd ed., p. 81.
STRASBURGER. 1900. Biolog. Centrbl. 20, 657.
VÖCHTING. 1893. Jahrb. f. wiss. Bot. 25, 149.
WIESNER. 1903. Biologie d. Pflanzen. 2nd ed. Vienna.
WINKLER. 1903. Ber. d. bot. Gesell. 21, 96.

LECTURE XXIX

FERTILIZATION, PARTHENOGENESIS, HYBRIDITY, HEREDITY

[In dealing with these phenomena we enter on a subject which, in spite of its great interest to physiologists, has been investigated almost solely from the morphological point of view. This renders its discussion very difficult as well as not infrequently onesided and subjective, since the main problems still await decision by experiment. In the additional notes in the present English edition only a selection of the recent literature can be dealt with.]

In plants, as in animals, there is, as we have already seen, a characteristic method of reproduction by the *fusion* of two previously separate cells, and in extreme cases the fusing cells, like the organs which produce them, are so widely different from each other that we may then speak of sexual differentiation and of the '*fertilization*' of a female by a male cell, in harmony with the conditions existing in the higher animals. In endeavouring to arrive at the *meaning* of fertilization we may consider the process as it occurs in definite cases among Archegoniatae and Angiospermae without inquiring whether fertilization has the same value in all cases. Since, doubtless, the sexual process has arisen not once only, but several times in the history of organisms, we must look on it as possible that in different places it may have a different significance, e.g. in Diatomaceae and Phanerogams (compare Klebs, 1899).

In certain Algae the principal difference between egg-cell and sperm lies in the much greater *size* of the former ; but both the gametes are normal cells, each has protoplasm and a nucleus, and each may even possess chromatophores. But in Phanerogams and ferns nearly all the protoplasm of the male cell disappears and the cell consists finally of little more than a nucleus. The protoplasm is never entirely absent, but, as far as we know, in the higher plants, the chromatophores are always wanting. We may therefore assume that the nucleus of the sperm is a most important organ, and is responsible for the final results of fertilization.

During the process of fertilization the protoplasts of the two cells unite and the two nuclei also undergo fusion, and thereafter the fertilized egg begins to grow. Without fusion neither male nor female cell is capable of growth. Thus the first result of fertilization is the removal of a developmental inhibition, and we may conclude that the egg was in *want* of something which is supplied by the sperm, and that its *nucleus* is destitute of *certain substances* which the male nucleus possesses.

It is probable that further research may show a qualitative difference to exist between the two nuclei, although at present such differences have not been determined. On the contrary research has hitherto shown a similarity in the materials composing ovum and sperm nuclei, and amongst these nuclein has been regarded as most worthy of attention. Although Zacharias (1901) in general found no qualitative difference in the nuclein present he found a considerable quantitative difference—the ovum-nucleus being much poorer in nuclein than the sperm-nucleus. In certain cases, for instance *Marchantia*, this difference becomes qualitative for *no nuclein* at all can be detected in the egg-nucleus just previous to fertilization. On this has been founded the hypothesis that the *ripe ovum is incapable of development because it does not contain sufficient nuclein, a deficiency which is supplied on fertilization ; but that the sperm cannot develop by itself because it is deficient in protoplasm.*

Views entirely different from that just advanced are held, two of which may be briefly referred to here ; one is based on the phenomena exhibited by the nucleus of the cell preceding the formation of the sperm and ripening of the ovum—phenomena resembling those seen in animals. It has often been remarked that the formation of male and female cells begins with a nuclear division differing widely from the typical mode, and described as *reduction-*

division. In the normal vegetative division the chromosomes, which occur in a definite and, for each species, perfectly constant number, split longitudinally, so that each daughter-cell contains the same number as the parent-cell. In the division which precedes the formation of the sexual-cells a similar number of chromosomes appears, but since they do not split longitudinally, both ovum and sperm contain only half the usual number and only by fertilization is the full complement again attained [compare Fig. 87 in the 7th ed. of the Bonn Text-book]. A phenomenon similar to this *reduction* is also found in Phanerogams. The nucleus in the embryo-sac and that in the pollen-grain show only half as many chromosomes as do the vegetative cells. The reduced number is not brought about, however, by suppression of the longitudinal division but by the nuclein thread dividing into only half the regular number of chromosomes. [Since these words were written a very comprehensive summary of the cytological literature has been published, showing that a reduction-division occurs before the formation of sexual-cells both in plants and in animals (compare KÖRNICKE, 1904; STRASBURGER, 1904 a, 1905, &c.]

Among zoologists the arrest of the developmental process has been attributed to this reduction, and many botanists, e.g. JUEL (1900 b), hold the same view as to the reduced number of chromosomes. Apart altogether from the fact that the reduction in the number of chromosomes is brought about in quite different ways there are other considerations which militate against this view. In speaking of the genetic relationships between Pteridophyta and Phanerogams in the foregoing lecture we saw that the whole of *one* generation of the fern—namely, the prothallial generation, on which the sexual organs are borne—is reduced to a few cell divisions in the spores of the Phanerogams. In ferns the prothallial generation possesses the half number of chromosomes and yet it is capable of vigorous development. Chromosome reduction in the Phanerogams is manifestly an ancestral character handed down from earlier types—perhaps fern-like in character—and has no direct relation to fertilization, as appears to be the case in animals. Also in the case of apogamy in ferns, in which the sporophyte arises on the prothallium without the intervention of an ovum it seems that the increase in number of the chromosomes can come about otherwise than by fertilization, for it can hardly be doubted that apogamous shoots have the same number of chromosomes as the normal one. In apogamous ferns, according to FARMER, MOORE, and DIGBY (Proc. Royal Soc., 1903, 71, 453), a fusion between the nuclei of two vegetative cells takes place, leading to a doubling of the chromosomes, and the nucleus resulting forms the starting-point for the apogamous growth. We ought, perhaps, to receive this statement with caution, and may raise the question whether the authors determined that such a fusion takes place *only* in apogamous prothalli, and also that such a fusion actually does serve as the starting-point for the new growth. Can the passage of a nucleus from the one cell to the other have been the result of the mode of preparation as in MIEHE'S observations quoted on p. 170? [FARMER has as yet given no further explanation, but DIGBY (1905) has published some additional preliminary notes to which reference may be made. NĚMEC (1904) also appears to incline towards the interpretation we have given above of the significance of the transference of the nucleus.]

For other consequences of the chromosome hypothesis we must refer to p. 377, and turn now to the consideration of another theory of fertilization which finds its chief advocate in BOVERI (1902). This hypothesis has grown out of investigations carried out upon animals. The egg is supposed to be incapable of growth because it possesses no '*centrosome*', and the influence of the sperm depends not on its bringing a nucleus but a centrosome to the ovum. Hitherto we have not spoken of this organ of the cell, simply because it is entirely wanting in the highest plants. The animal cell, on the other hand, very generally contains small granules surrounded by protoplasm exhibiting radiating

striae. These granules are capable of division, and one is found temporarily at each pole of the nuclear spindle. The idea is now very generally accepted that these centrosomes play an important part in nuclear division, since they determine the arrangement of the nuclear spindle and fix the dynamic centres, at least certain observations of BOVERI may be interpreted in this way. If then the egg forms no centrosome at all, or no *active* centrosome, then we can easily understand how its power of division is inhibited and how *perhaps* it cannot even grow. But BOVERI (1902) has not taken into consideration the fact that his theory will not hold in plants, where centrosomes are absent. If we follow STRASBURGER (1900a) in assuming that in the higher plants the centrosome may be represented by a partly-defined portion of the protoplasm, the so-called *kinoplasm*, we may extend BOVERI's hypothesis to the plant world also.

One thing is certain, viz. that none of the theories mentioned above can explain all the facts. All three agree in supposing that something is *wanting* in the egg, and so far all these hypotheses are correct. The phenomena of *parthenogenesis* however prove that this conception is not universally correct. True parthenogenesis, that is to say, the development of an unfertilized egg into an embryo, is not often met with in plants. Where it has been carefully investigated, as in *Antennaria alpina* (JUEL, 1900 a), and in species of *Alchemilla* (MURBECK, 1901), it has evidently become the normal process for the propagation of the species, and it is questionable whether the ovum in these plants is capable of being fertilized at all. The question would well repay an investigation, for if normal pollen does not occur in the species in question the experiment should be made with that of another closely-allied species. The investigation should prove of special interest, since JUEL has found that in *Antennaria* the egg contains the full complement of chromosomes present in the somatic cells, and he holds, for *this* reason, that fertilization is impossible. If KERNER's belief that *Antennaria hansii*, Kern., is a hybrid between *A. alpina* ♀ and *A. dioica* ♂ be well founded, then JUEL's position will be untenable (compare FOCKE, 1881, 194). [Meanwhile STRASBURGER (1904b) has shown in the case of *Alchemilla* and JUEL (1904) in the case of *Taraxacum*, both of which are parthenogenetic, that no reduction takes place in the formation of the ovum. The frequency in change of view on this subject and the contradictory nature of the observations make it necessary to proceed with caution. The fact established by OSTENFELD (1904) that certain species of *Hieracium* can produce embryos both sexually and parthenogenetically is of the greatest interest. Since it is unlikely that there are in these plants two kinds of ova we are bound to assume that the normal ova in the higher plants may develop parthenogenetically just as easily as those of *Marsilia*, and that the number of the chromosomes has not that significance which is attributed to it by the cytologists.] So far as the question that principally concerns us here is concerned these two plants are unimportant. Important on account of their bearing on the present question are certain species of *Marsilia* of which more than one shows a tendency to parthenogenesis. As NATHANSOHN (1900 a) has shown, it is possible, by raising the temperature, to increase very considerably the percentage of unfertilized eggs which develop. In one investigation on *Marsilia vestita*, at 18° C. only 1·3 per cent. developed parthenogenetically, at 35° C. 7·3 per cent. developed. The egg has the capacity, whether fertilized or unfertilized, of developing into an embryo, and in the latter case it is not the entry of a *material* substance but a rise in temperature that supplies the requisite stimulus. We must realize therefore that the stimulus may be supplied by a rise of temperature, just as by material contained in the sperm. That this material need not necessarily be nuclein or an organized portion of the cell, such as a centrosome or chromosome, has been shown by the experiments of LOEB (1899–1902) and WINKLER (1900) on animals. The ovum of the sea-urchin, which in nature only develops after fertilization, was treated by LOEB with magnesium chloride, and by WINKLER with a watery

extract of sea-urchin sperms, containing no nuclein, and as a consequence of this treatment the ovum developed.

We thus arrive at the conclusion that the egg needs a *developmental stimulus* before commencing to divide and grow (compare SOLMS, 1900, and the literature cited there), and such developmental stimuli we have already found to be very widely distributed. Thus we have seen that buds whose developmental activity has been temporarily inhibited may be made to unfold by ether, that the spores of many mosses germinate in the dark only when the temperature is raised, and that high temperatures are quite generally essential in propagation by cuttings. It is also known that the pollen-tubes in many cases induce development, apparently not by fertilization but by the secretion of some soluble material; thus the seed initials in Orchidaceae develop only if pollen-grains germinate on the stigma. [The same is true of *Fritillaria persica*, according to STRASBURGER (1886).] And this stimulus, doubtless chemical in its nature, may, according to TREUB (1882), also be effected by certain insects in one of the tropical orchids. In addition to this, the germination of the pollen-tube has an exciting influence in the development of the fruit. This is particularly noticeable in certain cultivated plants, which, as for example currants and Sultana raisins, produce no seeds, the ovules having degenerated. If the stigmas of these plants be not pollinated, the fruit fails to develop, but pollination causes development without leading to any fertilization (MÜLLER-THURGAU, 1898, compare also NOLL, 1902 [MASSART, 1902]).

Sufficient examples of the renewal of development as a result of stimulus action have now been given to show that the special effect of sperms is not without analogies. This is probably the most appropriate place to discuss certain phenomena which appear in the embryo-sac. We have comparatively recently learned that in Phanerogams not only does *one* sperm-cell from the pollen-grain fuse with the ovum, but that the *second* sperm-cell also passes into the embryo-sac and fuses with the united polar nuclei before these give rise to the endosperm (NAWASCHIN, 1898; GUIGNARD, 1899). As to the phylogenetic meaning of this second act of fertilization we need not here speak. We are only concerned at present with the fact that we appear here to be dealing with the removal of a developmental check. Apparently *all* the nuclei of the embryo-sac are incapable of development without some developmental stimulus, but the requisite stimulus is not always a nuclear fusion. There is a whole series of plants in which the embryo, as in apogamous ferns, arises not from the ovum but from neighbouring cells (compare ERNST, 1901), it may be from the synergidae, the antipodal cells, or from cells of the sporangium wall external to the spores—i. e. from the nucellus. In the last case especially a fusion with a male cell is entirely out of the question. But a definite external stimulus is in many cases necessary for the formation of such adventitious embryos. In *Nothoscordon fragrans*, for instance, the adventitious embryos first appear only after the ovum is normally fertilized. After recent discoveries a reinvestigation of the question is much to be desired, all the more as in certain other cases where adventitious embryos occur, e.g. *Coelobogyne ilicifolia* (STRASBURGER, 1878) and probably also *Euphorbia dulcis* (HEGELMAIER, 1901), they certainly arise without the previous action of pollen-tubes or sperm-cells. All stages, from normal embryo formation to the complete parthenogenesis of *Antennaria* and *Alchemilla*, and the adventitious embryo formation in *Coelobogyne*, occur in nature. In the last-mentioned cases we must look for an internal stimulus which initiates the development of the cells concerned.

The above examples of adventitious embryos are also of interest from another point of view. It appears that all cells contained in the embryo-sac or which come to lie therein, assume a form similar to the normal embryo; the embryo-sac cell must be able to exert a similar stimulus (JUEL, 1900 a; STRASBURGER, 1878).

We may now return to the phenomena of normal fertilization. We have

seen that the egg requires a developmental stimulus and we may suppose that the sperm-cell by itself, perhaps only on account of its poverty in protoplasm, perhaps also for other reasons, is incapable of development. Bearing on the first of these possibilities is the phenomenon of 'merogony', which has been examined in both animals and plants (ROSTAFINSKI; WINKLER, 1901). By appropriate means, portions of the egg containing no nuclei were detached and fertilized. The sperm-cell when embedded in a considerable mass of protoplasm commences to develop. This shows that the sperm-cell when enclosed in protoplasm derived from the egg-cell becomes capable of development and undergoes 'andro-' or 'ephebo-genesis'; we may indeed give it another meaning and say that the nucleus of the ovum is *unnecessary* to development, and that it is sufficient if a sperm-nucleus is added to a non-nucleate egg.

Moreover, there is also a question of fundamental importance to be faced, viz. *why are the two sexual-cells incapable of development on their own account? Is the inhibition of development due to internal causes? Is it a result of old age and is the fusion to be regarded as a case of rejuvenescence?* This latter hypothesis has often been suggested, although not proved, though sound arguments against it are not forthcoming. We must content ourselves therefore with a a reference to what has been said already against the need for rejuvenescence, adding only that the means employed is extremely peculiar. It is certainly by no means clear how by the fusion of two senile units a rejuvenescence can result—one might just as well expect that such a fusion should lead to increased senility. If we can allot a more suitable meaning to the fusion, then the idea of rejuvenescence as resulting therefrom may be readily given up.

In recent years another interpretation of the process has become more and more prominent and has now received very general support, namely, that the fusion of the two cells in the act of fertilization is of primary importance, inasmuch as it unites the characters of two organisms. In asexual reproduction, i.e. by means of spores in Algae and Fungi, a cell is set free from the mother-plant, and from this a new organism is produced with characters similar to those of the parent. The spore thus transmits the characters of the ancestor to the offspring, the latter *inherits* the ancestral peculiarities. If now in two plants or in two branches of the same plant differences of some kind exist, then these differences—at least under certain conditions—may be transferred to the offspring. In asexual reproduction, which we may describe as *monogeny*, individual peculiarities may thus remain unaltered. Let us now assume that in sexual reproduction (*digeny*) each cell carries potentially certain individual differences derived from its parent, or, as it is usually put, has in it *initials* of these characters, then these initials are united and mixed in the fertilized egg. Hence we may, with WEISMANN (1892 b), term fertilization '*amphimixis*'. So far, there is a general concurrence of opinion amongst authors that this *union* of characters is the predominant feature in fertilization, but whether the significance of the union be the *balancing* of individual characters or whether by a blending of the two organisms *new* characters *originate* is still a matter of discussion.

We must now attempt to discuss these two possibilities more closely, and first of all it may be emphasized that in such an interpretation of fertilization the observed inhibition in the development of the sexual-cells at once attains a new significance. It is to be regarded as an adaptation, which in the first instance renders fusion possible. For if the egg or sperm surrounded itself with a cell-wall immediately after its formation and then began to grow, any fusion of the protoplasms and nuclei of the two cells would be impossible.

We must not, however, overlook the fact that the above views do not harmonize with all the facts we recognize under the term fertilization. If the swarmspores formed by division in *one* cell of an alga copulate in pairs on swarming free from the mother-cell (p. 354), the differences between these cells can hardly be so great that a mixing has any very special significance. The

sexual-cells also which arise from stamens and carpels of one and the same flower can scarcely possess any very marked individual characteristics in their initials. Still, it is known that there are numberless arrangements to be observed in flowers by means of which 'self-pollination', that is to say, transference of pollen to the stigma of the same flower, is prevented, and by which cross-pollination between neighbouring branches or even between neighbouring plants is facilitated (Cross-fertilization, DARWIN, 1876). Moreover the gametes formed in one gametangium in certain Algae are said not to fuse with each other (STRASBURGER, 1900 b, 306).

If we now try to follow up more closely the complete combination of initials in fertilization we at once come face to face with a great difficulty, for systematic experiments are wanting in which well-chosen examples with marked individual characteristics have been crossed and the offspring thoroughly studied. To carry out such researches would be very difficult, since individual differences in plants are not, as a general rule, very well marked. For this reason we must go further afield and study hybrids between different plant relations (races, varieties, species) which show more easily recognizable differences between each other. As to such crosses there exists a voluminous literature. The existence of such races we take for granted—the next lecture will deal with their mode of origin—in the present lecture we will deal only with the results of crossing individuals belonging to two different races—that is to say, with the production of *hybrids* (the older literature relating to these will be found in FOCKE, 1881). [For more recent literature see DE VRIES, 1903.]

So far as we are aware, FAIRCHILD, in England, in the year 1717, was the first gardener to raise a hybrid, inasmuch as he pollinated the stigma of *Dianthus caryophyllus* with pollen for *D. barbatus*. Among botanists KÖLREUTER (1761) was the first to conduct experiments in hybridization for years on a large scale. He was interested in hybrids from the point of view of their bearing on the sexuality of plants, a subject which had been much disputed. His first hybrid, *Nicotiana rustica* ♀ × *N. paniculata* ♂, flowered in the summer of 1761. Since then innumerable hybrids have been produced for scientific and horticultural purposes; many of them have arisen naturally, and yet recent developments in our science indicate that we have only just crossed the borders of the subject, and that in this province a wide and interesting field of research lies open to us (compare DE VRIES, 1900; CORRENS, 1900 onwards; H. TSCHERMAK, 1900; summarized by CORRENS, 1901 a and 1903, [1905]).

It is not possible to produce hybrids from any two plants selected at random, because the capacity for forming hybrids is generally restricted to nearly-allied plants. Rarely do we find species of different genera capable of hybridizing; less frequently still are hybrids themselves capable of crossing. The more closely related the plants, the easier it is, as a rule, to produce a hybrid. Still, the capacity for hybrid-production does not, in any sense, run parallel with systematic relationship. It is very noteworthy that in certain cases the hybrid *A* ♀ × *B* ♂ may occur while the reciprocal *B* ♀ × *A* ♂ is impossible. Thus *Mirabilis jalapa* ♀ is easily crossed with *M. longiflora* ♂, whilst it is impossible to fertilize *M. longiflora* ♀ by *M. jalapa* ♂. Such a fact at first sight seems almost incomprehensible, but it is suggested that the success of the cross depends not only on the capacity of the sexual-cells to fuse with each other but on the possibility of the approximation of these cells. It is usually assumed that the pollen-tube of *M. jalapa* is too short to grow through the much longer style of *M. longiflora*. This may be true, but there is also another possibility, recently drawn attention to by BURCK (1900). BURCK found that many stigmas contain substances which are capable of stimulating the pollen-grains only of some, but not of all, species of the same genus to develop. It must be assumed that the failure of many experiments in crossing is due only to the fact that the pollen-grains of the male parent are incapable of germinating on the stigma of the

other plant. It is possible that by transferring a drop of the substance secreted by the stigma of the pollen-bearing parent to the stigma of the other plant germination of the pollen-grains may be induced.

When the hybridization is successful seeds are formed which resemble in form, colour, and size the normal seeds of the mother-plant; the fruits also are uninfluenced by the male parent. A variation can ensue only where the contents of the pollen-grain come immediately into action; that is to say, where the two generative nuclei have fused with the essential nuclei of the embryo-sac, i.e., the endosperm on the one hand, and the embryo and the plant arising from it on the other. At present we must omit any consideration of the *endosperm* of the hybrid, and deal only with the *hybrid* proper. No general rule can be laid down as to the appearance of the hybrid. Many interspecific hybrids show a structure exactly intermediate between that of the two parents, as KÖLREUTER described it in the case of the first recorded 'botanical cross'—the hybrid *Nicotiana rustica* ♀ × *N. paniculata* ♂:—'I was gratified to find that the hybrid took a median place between the two parents not only in the arrangement of the branches, and in the position and colour of the flowers, but also in all the parts of the flower (the stamens alone excepted), which exhibited an almost geometrical mean.'

Horticulturists, in their researches on hybrids, usually start with the assumption that the hybrids will show characters intermediate between those of the parents, but it is now becoming more and more evident that this is only one of many possibilities. In hybrids between members of closely-related races intermediate characters are often *wanting*; for instance, the hybrid obtained by crossing a red with a white-flowered pea is not light red, but red, like one of the parents. Although the white-flowered pea has yellow cotyledons and the red-flowered one has green, the hybrid has *yellow* cotyledons. From this example it is clear that in the hybrid the *one* parent does not simply predominate over the other, but that the *separate characters* of the two parents struggle with each other, one parent being eventually victorious with regard to one character the other in another. In this connexion we may, with CORRENS, speak of *dominant* and *recessive* characters, and we may designate hybrids with such strongly-marked characters as heterodynamic, in contradistinction to homodynamic forms, which exhibit characters more or less exactly intermediate. A further complication not infrequently arises, viz. in certain hybrids it cannot be determined once and for all which character is dominant and which recessive; the result may be different in each individual case where the characters are brought into conjunction. Individuals resulting from a single bastard cross-pollination may differ, and in one or in all characters may resemble at one time the mother at other times the father (many species of *Hieracium*, MENDEL, 1870), or some of the hybrids may partly resemble the mother only or partly the father only (strawberry, MILLARDET, 1894). Finally, in other cases still, the decision as to which character shall dominate, or how vigorously it shall show itself is by no means determined by fertilization itself, for individual branches, tissues, cells, or even cell-parts belonging to one and the same hybrid may show variations. Examples of such 'mosaic hybrids' were found by NAUDIN (1862) and in the hybrid *Datura laevis* ♀ × *D. stramonium* ♂, which produced, in addition to fruits with small spines, intermediate, therefore, between the large-spined *D. stramonium* and the spineless *D. laevis*, fruits which were smooth on one side and spiny on the other.

Although the hybrid at first sight often appears as a new creation, more careful study shows that it is only the *combination* of characters in it that is new; absolutely new characters do not seem to arise in hybrids, although there are certainly exceptions. The hybrid between the green-stemmed, white-flowered species *Datura ferox* and *D. laevis* has brown stems and violet flowers; it would appear in this case as if a new character, the formation of a new pigment, had

arisen. In reality, however (WEISMANN, 1892 a, p. 421), the phenomenon may be otherwise explained. The white-flowered species may have arisen from a violet-flowered stock and may, so to speak, have the capacity for forming this colour in a *latent* state (compare p. 376), but which in the hybrid becomes actual. Whether all the unexpected colours which appear in hybrids are to be explained in the same way must remain at present an open question. A much more frequent variation of the hybrid from the parents lies in this, that the hybrid, as a rule, differs in its *growth-energy*. This energy may be feebler than that of the parent plants when the parents are not closely related forms. In this case the seeds germinate badly and the seedlings are difficult to rear. Or—and this is particularly applicable to hybridization between nearly-related races—' they are remarkable for their size, rapidity of growth, early blooming, free flowering, longer period of life, great capacity for multiplication, abnormal size of individual organs, and similar characters' (FOCKE, 1881, p. 475). If, for instance, the hybrid *Datura tatula* ♀ × *D. stramonium* ♂ attains a height of two metres, while the parents only attain a height of about one metre, we may say that the hybrid has acquired a new character, nevertheless it is only a quantitative and not a qualitative variation such as we might obtain otherwise, e.g., by over-nutrition in seed-formation or good manuring in germination. One may look just as little upon the increased growth-energy of the hybrid as on the other, at all events, frequent characteristic, its diminished *fertility*, as a serious objection to the view that hybrids show no new characters. This diminished fertility is usually manifested in partially or completely unfertile pollen, more rarely in immature ovules. For this reason it is often possible to rear fruits and seeds only by pollinating from the parental line, although there is a class of hybrids whose ovules are quite fertile with their own pollen (*Salix*, *Hieracium*). At the extreme limit of sterility are many species of *Rhododendron*, *Epilobium*, &c., hybrids of which, in general, do not even form flowers (compare p. 376).

Let us now inquire what the hybrids of the second generation look like, that is to say, the plants arising from the seeds produced by the first hybrid generation. In this case, even less than the other, no general rule can be laid down, even leaving out of consideration self-sterile hybrids. Among fertile hybrids there are doubtless those in which the offspring after self-fertilization are quite similar to the parents (MENDEL'S *Hieracium* hybrids, CORRENS, 1901 a, pp. 75, 80) and, in contrast to these forms, those in which the offspring are entirely different. [Owing to the discovery of parthenogenesis in *Hieracium* the uniformity of their offspring appears in an entirely different light; there are hybrids, however, which are perfectly constant (DE VRIES, 1903, 66).] Much work has recently been carried out on the latter class of hybrids, and the numerical relationships of the individual variations have been worked out on MENDEL'S principles. In experiments of this kind, all, or at least very many, of the seeds produced must be sown, but in the earlier investigations on this subject only a few plants were raised, and hence no conclusions as to the constancy or inconstancy of hybrid progenies could be arrived at. We cannot do more than give one example from the very voluminous literature on the subject. Let us consider a pea-hybrid that has arisen from two races differing in one character only, say the colour of the flowers, which in race *A* are red, in race *B* white. As we have already seen red is the dominant character. All the hybrids of the first generation have *red* flowers. If the seeds produced by self-pollination from these hybrids be raised, the majority of the offspring will be found to produce red flowers, but a certain proportion will be white-flowered. An enumeration shows that 25 per cent. are white-flowered and 75 per cent. red-flowered. All the offspring of these white-flowered plants remain white-flowered, while of those of the red-flowered forms one-third remain unaltered in colour (red) while of the other two-thirds 25 per cent. are white and 75 per cent. red. In order to explain this extremely peculiar result MENDEL (1866) assumed that the

hybrid possessed two sets of reproductive cells, initials of the red-flowered form and initials of the white-flowered form, in equal number. Fusion between two sexual-cells with the same initials might then be as frequent as fusion between sexual-cells with different initials. In 100 fertilizations we shall get on an average fifty cases of fusion of similar initials—twenty-five fusions of cells bearing the initials of white with white, twenty-five fusions of red with red—while fifty fusions of dissimilar initials might also take place. Whether the red fuses with red, or red with white is immaterial; since red is dominant, 75 per cent. of the second generation will show red flowers, and only the 25 per cent. in which white has united with white will exhibit white flowers. But these 25 per cent. have for ever lost the power of producing red flowers, and in this fact lies the evidence for the support of the theory that the sexual-cells of the hybrid contain only *one* kind of initial, while the vegetative-cells contain both types of initial. There is thus in the formation of sex-cells a *segregation* of the initials. Amongst the 75 per cent. of red-flowered forms only 25 per cent. have red initials, and these are marked off from the fifty others which carry white also, when sexual-cells are first formed. The twenty-five again form *only one kind* of sex-cell, while the remaining fifty again segregate. Let us imagine a case in which each plant produced only four offspring. We may then construct the following scheme of the numerical relationships between the forms with white, red, and mixed (red + white) *initials* in five generations.

Generation I.	Generation II.	Generation III.	Generation IV.	Generation V.
	1 white →	4 white →	16 white →	64 white
		2 white →	8 white →	32 white
			4 white →	16 white
				8 white
1 red + white	2 red + white	4 red + white	8 red + white	16 red + white
				8 red
			4 red →	16 red
		2 red →	8 red →	32 red
	1 red →	4 red →	16 red →	64 red

Thus we see that the 'white-flowered' character, lost apparently in the first generation, reappears in the second generation in 25 per cent. of the forms, and rapidly increases, until by the fifth generation there is but little difference in number between the red and the white-flowered individuals.

MENDEL'S *law of segregation* is, however, not of universal application. There are hybrids which do not segregate and also others which segregate in different proportions. Segregation may occur in *one* character while another does not segregate. The segregation or non-segregation of characters does not depend on whether the characters in the formation of hybrids are homodynamous or heterodynamous. Notwithstanding the great interest attached to the behaviour of races differing in two or more characters we must content ourselves with what has been already said, and in conclusion raise the question as to how far these facts suggested by the study of hybrids bear on the general problem of fertilization.

Bearing in mind the increased power of growth of many hybrids and DARWIN'S statement that seeds derived from cross-fertilization produced more vigorous seedlings than those resulting from self-fertilization, it may be said that cross-fertilization brings about a rejuvenescence or renewal of youth in the protoplasm. Further, we may assume that protoplasm without such rejuvenescence may finally become, by continued vegetative reproduction, senile. Although it is certain that the hybrid frequently exhibits more vigorous powers of growth still any conclusion founded on that fact is insecure. It has not been shown that the increased vigour depends on the *fusion of the two kinds of initials* from which the *hybrid* results, and it seems almost more probable that the very stimulus which removes from the egg its inability to develop may be also

the cause of the more vigorous growth of the hybrid. This releasing stimulus may reside in a soluble chemical compound. We have already often spoken of stimulation of growth by 'poisons', and we may compare the poisonous effects of the action of copper-sulphate and other chemicals with the effect of hybridization, for there, as in hybrids, we may often have, side by side, an increase in vegetative growth and an inhibition of the formation of reproductive organs (compare pp. 88 and 374). Such facts as these, viz. that widely separated races produce weak-growing hybrids, that more widely separated forms do not hybridize at all, and that the pollen of a race still more distantly related may even injure the stigma of another form, recall at once the action of poisons [comp. DARWIN, 1868, II, 180].

In addition to the incentive to growth, fertilization leads to the fusion of two different protoplasts, each having their individual initials. What do hybrids teach us in this relation? It is perfectly obvious that such a combination occurs but we have nothing to go upon to enable us to determine what will be the primary result of this union. On account of the *varied* behaviour of the hybrid we can say nothing as to whether a form will arise *intermediate* between the two individuals, whether the differences will be neutralized and the *species remain constant* or whether on the contrary new types will appear and maintain themselves and the species become *polymorphic*.

Although hybrids do not at present throw light on the problems as to the significance of fertilization, they are nevertheless of primary importance in another important question, i.e. heredity, the phenomenon of the handing on of the parental characters to the offspring. Heredity is a peculiarity of organisms which is shown both in the simplest form of reproduction, i.e. fission, and in the most complicated sexual process, but although in the first case it may be regarded as a matter of course, in the latter it is an extremely wonderful phenomenon. Considering, e.g., division in the cell of *Spirogyra*, the protoplasm, the nucleus, and the chloroplasts divide, and these products of division have the power of growth. Now if new characters appeared in these two halves which were not present in the parent it would be a much more wonderful fact and one much more difficult of explanation than if each half had the peculiarities of the whole as is, as a matter of fact, the case. If we consider now a complicated plant, such as a mushroom or a moss, we find there single cells—the spores—capable of giving rise to a new organism similar in all respects to the parent. In each spore the *initials* of the whole organism must be present and there must also be arrangements for the development of these initials in the way characteristic of the organism in question. Looking at sexual reproduction only we have seen clearly from our study of hybrids that in *both* sex-cells the initials of an entire organism are present, and hence that the fertilized ovum contains the initials of *two* organisms—although only *one* results. Since in certain cases it may be clearly proved that these initials do not unite later on, we arrive at the conviction that in each sexually-produced plant there are many initials which do not develop but remain *latent*.

It is impossible for us at present to deal with the problem of heredity in all its bearings, all we can do is to treat of it briefly in so far as it is elucidated by fertilization and hybridization. Let us direct our attention in the first place to the question of the material basis of heredity.

In the division of *Spirogyra* no special theory need be referred to. All the organs of the daughter-cell are parts of corresponding organs of the mother-cell; the nucleus inherits qualities of the nucleus, the chloroplast those of the chloroplast, and so on. Let us compare with this case the development of the flowering plant from the ovum, and confine ourselves at first to the individual *cells* which arise from it. The protoplasm and the nucleus of each of these are also derivatives of the plasma and the nucleus of the egg, and we may therefore admit at once that all the peculiarities of protoplasm, and all the peculiarities of the nucleus

are inherited from the parent protoplasm and nucleus respectively. In the chromatophores, however, we meet with difficulties at the very outset. So far as we know, only the egg possesses these chromatophores, the sperm-cell has none. Should a more thorough examination lead to another result—which is quite possible—we should expect the chromatophores to fuse during fertilization just as the nucleus and protoplasm do. According to the present point of view, however, either the peculiarities of the chromatophores of the father are incapable of being inherited, or they must be passed on by another portion of the cell, e.g. the protoplasm or nucleus or both. The assumption that the inheritance of a character by part of a cell which was not itself the natural carrier of this feature cannot be avoided, since all the peculiarities of the cell are not associated with those organs of the cell which are capable of division and which increase by that means *only*. The wall of the vacuole with its contents and the external protoplasmic layer with its product, the cell-wall, are, for example, parts of the cell which are not directly transmitted from generation to generation. The capacity of forming such parts, which undoubtedly is possessed by the egg, is what is meant when we say that such a cell contains the initials of such organs. We may now inquire whether there is any evidence for the definite *localization* of these 'initials'. Although we do not really know what the 'initials' are, we must nevertheless assume that they are inherent in the *protoplasm*. It is not probable that any selected part of the protoplasm functions as the bearer of the initials, and yet since the time of NÄGELI (1884) it has been customary to designate as 'idioplasm' that part of the protoplasm which contained the initials and induces the rest to go through a certain development, and to describe the remainder of the protoplasm as trophoplasm. NÄGELI considered the idioplasm as a network which extended throughout the entire cell, although in recent times greater localization has been given to it.

On the zoological side (HÄCKEL, 1866) the *nucleus* is very generally claimed to be the bearer of hereditary characters, and of the idioplasm (compare HÄCKER, 1902), and on the botanical side, investigators, such as STRASBURGER (1884) and DE VRIES (1889), have supported this view or endeavoured to prove it independently. It may be pointed out, in the first place, that in the process of fertilization the male element consists of a nucleus with *little or no* protoplasm. That the idioplasm is localized in the nucleus has not satisfied every one, and other hypotheses have been advanced, such as, for example, that the *chromosomes* of the nucleus are the agents specially concerned in heredity. In this connexion the changes preceding division of cells and fertilization must be kept in view. In every normal cell division the characters of the mother-cell are equally divided between the two resulting daughter-cells, and so also the units which act as the bearers of the initials must be divided into exactly similar halves. This tallies with the well-known division of the chromosomes, where the division allots a longitudinal half to each daughter-cell. The bearers of individual initials are believed to be serially arranged in the chromosome, and to correspond to a certain degree with the chromatin-granules, which in certain cases may be seen with the microscope, and are separated from each other by linin-threads. These chromatin-granules, arranged longitudinally in the chromosome, are necessarily present in the resting nucleus, although they cannot be seen in it. The conclusion has been drawn, therefore, that the chromosomes are persistent organs of the nucleus which can only multiply by division, and moreover by longitudinal division, but which can never be created afresh. It is very remarkable that at every division the chromosomes appear in the same number, and their behaviour during fertilization in the animal kingdom is even more remarkable still. There the so-called reduction division (p. 367) comes into operation, ovum and sperm have only half the number of chromosomes found in the somatic cells, and so the doubling of the number of chromosomes at each fertilization is prevented. The fact that the sperm-nucleus contains the same

number of chromosomes as the nucleus of the ovum is regarded as a proof that the chromosomes are really the transporters of hereditary characters, for in general the offspring inherit in equal degree from father and mother.

We have hinted at the basis on which certain modern theories of heredity are founded, and more especially WEISMANN'S germ plasm theory (1892 and 1902), although their characteristics have by no means been treated exhaustively. WEISMANN'S theory is at least the first theory of heredity to be worked out in detail, and leads to results of the greatest consequence. It is for this reason worthy of consideration and its scientific importance is greatly enhanced when we reflect how many specific researches it has given rise to. If we do not deal with it here *in extenso* it is not merely from considerations of space, but also on its merits, for botanists must, in our opinion, reject this theory. Detailed reasons for rejecting WEISMANN'S theory we cannot give here, all we need say is that to attribute to the chromosomes, or indeed to the nucleus at all, the exclusive possession of the initials is a view which has in no sense been justified.

Let us first of all consider the question of the individuality of the chromosomes. [The principal supporter of the individuality of the chromosomes of late years has been BOVERI (1904), for STRASBURGER, who also held that view in the past, appears to have abandoned it (1905). The parts of the chromosomes, the chromatin-granules or 'ids', are still to be considered as organs which may increase in number by dividing, but which cannot be created afresh.] In the majority of nuclear divisions an unprejudiced observer will conclude that the chromosomes are formed in the so-called prophase stage of the division and will disappear in the anaphase. The constant return to the same number of chromosomes results from the fact that before each division the mass of chromatin is approximately of the same amount. There are statements enough available which suggest that the constancy of the number of chromosomes is often more a pious wish on the part of the observer than an actual scientific fact. Very frequently the number of chromosomes cannot be accurately counted at all, and the observer contents himself with ascertaining whether they correspond *approximately* to the normal number, or to its half, or its double. GUIGNARD (1891) adduces an example of a very remarkable anomaly in the number of the chromosomes. The primary embryo-sac nucleus of *Lilium* has in its first division twelve chromosomes; one of the resulting daughter-cells always exhibits in the two following divisions twelve chromosomes, the other and lower one, however, shows, in the first division, sixteen or more, and in the second twenty to twenty-four. This, at all events, proves that the chromosomes can reproduce themselves otherwise than by longitudinal division. DIXON (1894) has made similar observations in the prothallium of *Pinus*, where the large nuclei of the archegonium-wall cells possess more than double the number of chromosomes found in the nuclei of the first prothallial cells. Finally, we may draw attention to the increase in the number of chromosomes in apogamous ferns (compare p. 368). In spore formation also, as above explained, a diminution in the number of the chromosomes arises, not, as one might assume, because, as in the case of animals, the longitudinal division fails to appear, but because the chromatin of the nucleus breaks up into only half the number of chromosomes. We conclude from all this that the chromosomes are not definite *organs* of the cell as are the chromatophores and the nucleus; they are reformed at each division, and hence the chief basis for believing them to be transmitters of hereditary characters disappears.

Starting from the fact that the internodal cells of the Characeae are incapable of regeneration, it has been claimed that only by normal nuclear division can two cells with equal hereditary properties arise. The above-named internodal cells show later, as a matter of fact, the so-called direct cell division, in which no chromosomes and consequently no longitudinal division of the chromosomes can be found. When *first produced*, however, the nucleus divides in a thoroughly typical manner, and the sister cells at the nodes possess fully the

quality of regeneration. The later indirect nuclear divisions in the internodal cells may possibly be the *result* of the loss of capacity for regeneration, but could never be its *cause*. Furthermore, NATHANSOHN (1900 b) has shown for *Spirogyra* and WASILIEWSKI (1903) for *Faba* that by taking certain measures the typical nuclear division may be transformed into the direct method without the cells suffering any loss of function (compare p. 270). Besides this, there are plenty of cells which are unable to exercise this or any function, e. g. regeneration, but whose nuclei have nevertheless arisen in the normal manner. In a word, conclusive proof that the idioplasm is localized in the chromosome does not exist.

But the chromosomes do not constitute the whole of the nucleus, and perhaps the hereditary capacity lies in some other nuclear substance. The supposition that the nucleus serves *only* as a receptacle for reserve material connected with the hereditary substance must be entirely rejected, for in such cases as *Spirogyra* it would be quite superfluous. If, however, other functions than heredity be attributed to the nucleus, but if heredity is to be associated with it *only* and not with the plasma, we have before us a view to which we must take exception. This fact alone would appear conclusive, viz. that every male cell bears protoplasm in addition to the nucleus, but it does not seem permissible to place a value on its amount. Further, in the Phanerogams, STRASBURGER (1900 b), himself an advocate of the 'nuclear theory', has quite lately conceded the passage of protoplasm along with the male nucleus into the embryo-sac, although, it is true, he points out that it is difficult to recognize it there. GUIGNARD (1900, 373) has also seen protoplasm pass from the male cell to the ovum.

If BOVERI'S experiments were beyond criticism they would give extremely strong support to the nuclear theory. BOVERI fertilized the non-nucleate ova of one sea-urchin with the sperms of another, and obtained hybrids showing paternal qualities only. Unfortunately, grave exception has been taken to these experiments (compare A. MEYER, 1902, p. 173). [BOVERI, 1904, 105.] [GODLEWSKI (1905) has advanced experimental proof that a sea-urchin egg deprived of its nucleus may be fertilized by the sperm of *Antedon* (one of the Crinoideae), and that the organism resulting has the characters of the mother, whence it may be concluded that cytoplasm without a nucleus is capable of transmitting hereditary characters.] The question of the localization of the bearer of the hereditary characters must for the present therefore be left undecided, or else we must assume that this is to be sought for just as much in the nucleus as in the protoplasm, perhaps also in the chromatophores. Moreover, we are not only ignorant *where* this substance is to be found, but we are equally in the dark as to the *way* in which it *operates*.

Having examined the distribution of the idioplasm in the *cell*, let us now turn to its distribution in the plant as a whole. Here we find two sharply opposed views. One claims for the germ-cells (sex-cells and cells of growing points) a very special rôle, they alone are said to be the bearers of the whole idioplasm; the other theory assumes the same potentialities to exist in any and every cell. The first bases itself on the specialized phenomena in the animal world, the other on similar phenomena in the vegetable world. In many animals the egg by the first division is divided into two essentially different cells, one of which is devoted to the construction of the whole soma, the other to the formation of the sex-cells. The germ-cell, according to WEISMANN, contains the initials of many organisms; the somatic-cell of one only. By the further division of the somatic-cells there arise the rudiments of individual somatic organs which only develop further in this one direction. WEISMANN, therefore, assumes that unequally inheritable divisions occur, so that, for instance, in one cell there are *only* initials for the ectoderm, in another *only* those for the endoderm. Against this view we may urge the behaviour of certain plants, notably *Begonia* and *Marchantia*, in which undoubtedly from *every* cell, even when advanced in growth, the whole organism can be reproduced by regeneration (p. 330), and in which,

therefore, the vegetative-cell evidently contains the same initials as the germ-cell. Now there are, on the one hand, animals which are like typical plants in possessing extensive powers of regeneration, and there are also plants which are comparable with the higher animals in their slight powers of regeneration. How are such plants to be regarded ? Are the root initials absent in a twig which cannot strike root, and does a leaf which forms roots but not shoots, possess *only* root initials ? We can give no final answer to this inquiry ; but when we reflect how much regeneration depends on external conditions (e.g. high temperature) we shall be inclined to believe that only a favourable environment is wanting and not initials. Moreover, initials originally present may easily be lost or so very much attenuated in efficiency—*sit venia verbo*—by the growth of the cell that they become inoperative.

Regeneration can but rarely proceed, as in the case of *Marchantia*, from small fractions of the body of the plant ; isolated cells of fully-developed tissue are mostly only just capable of growth (HABERLANDT, 1902), or on the application of stimuli only produce a few divisions (WINKLER, 1902), but they never regenerate a complete organism. The possibility is not excluded that suitable nutriment is wanting in these cells such as would render possible an increase of the small quantity of idioplasm which they contain. If regeneration taking place in a tissue proceeds from a single cell (*Begonia* (HANSEN, 1881), *Torenia* (WINKLER, 1903)) these cells are always seen first to divide actively *without growing* ; WINKLER supposes that these divisions indicate an increase of idioplasm. Also in callus the formation of organs is always preceded by an ample division of cells, which WINKLER regards in a similar way, but nothing more definite is known. In any case the botanist may, in opposition to WEISMANN, maintain that all cells have the *same* initials, and that there is no qualitative division of hereditary characters. The germ-cells are distinguished from the vegetative-cells only by the fact that in them the idioplasm is predominant, while in the latter, in accordance with their function, trophoplasm prevails.

But how is this to be reconciled with the segregation of characters which appears in certain hybrids and which seems to be characteristic of the germ-cell ? Is not this segregation in itself a new argument for the chromosome hypothesis ? If every chromosome were the bearer of *one* quality, then the reduction division must be an excellent means of effecting qualitative division of hereditary characters, thus leading to segregation. The small number of chromosomes, however, renders it impossible to allot *one* quality only to each of them, and the theories which allot to the chromosome the hereditary substance are obliged to assume that each *chromatin-granule* is the bearer of *several* qualities. The reduction division as understood by the zoologist does not explain the segregation of the hybrids ; on the other hand, CORRENS (1902) has formulated a view which renders it possible to understand this process on the grounds of the chromosome theory. We will not pursue further these purely hypothetical questions, but confine ourselves to the facts, regarding it as established that the supposed distinction between somatic- and germ-cells does not in reality exist at all.

We have already referred to segregations which take place in the vegetative region, but one of the most instructive examples is furnished by *Cytisus adami*, a hybrid of *C. laburnum* and *C. purpureus*. This hybrid is approximately intermediate between its parents so far as its organs of vegetation and reproduction are concerned ; thus, for example, it produced flesh-coloured flowers. But reversions to the parental forms also occur, for on individual branches we frequently find the yellow blossom of *C. laburnum*, and also, but more rarely, the red flowers of *C. purpureus*. If a branch has assumed the character of one of the parents it never returns to the intermediate form. The segregation need not involve the whole branch ; it can also be accomplished in the longitudinal half of a bud, so that the branch arising from it has on one side the character of *C. laburnum*, and on the other that of *C. adami* ; and in this case the line sepa-

rating the two often passes through a flower or through a leaf (BRAUN, 1851). These segregations also claim our interest in that they are *not confined to the derivatives of individual cells, but present themselves at the same time in many cells* (BEIJERINCK, 1901). The segregation in this case would therefore not be directly connected with the *division of the cells*, and there must evidently be a subsequent destruction of initials in the fully-formed cells.

If initials, however, can disappear in a fully-formed cell it is possible that they may also arise in them. We have every reason for treating this question in connexion with *C. adami*. This plant, which passes for a hybrid, is said to arise from the *grafting* of *C. purpureus* on *C. laburnum*. As, however, an experimental production of this graft hybrid, after its first fortuitous occurrence, no longer succeeded, and as there appeared little probability of the formation of graft hybrids in any case, their existence has latterly been denied (VÖCHTING, 1892). The very definite statements of KÖHNE (1902) about a hybrid arising from a grafting of *Mespilus* on *Crataegus* would appear to render this sceptical point of view no longer quite justified. [According to NOLL's (1905) most recent statements it is scarcely possible to doubt that in this case we are dealing with a genuine graft hybrid.] The hybrid twigs arise in this case at a certain distance from the point of grafting, and it therefore appears quite impossible that they could have arisen from cells which have grown together in the process of grafting. [NOLL considers that such a fusion of nuclei is self-apparent.] It must rather be an effect produced by the *Mespilus* cells on distant *Crataegus* cells. As STRASBURGER (1901 a) has found plasma bridges between graft and scion, a migration of plasma particles, therefore, also of idioplasm, from the graft into the scion is not impossible. No one, however, will wish to maintain that cell-nuclei wander through these plasma bridges and unite with the nuclei of other far distant cells, for the observations we owe to MIEHE (1901) and KÖRNICKE (1901) regarding the wandering of nuclei from closed cells refer evidently to pathological processes or artificial products. [In any case we must take exception to NĚMEC's (1904) and FARMER's (1903) statements that a nucleus which has migrated from one cell can fuse with another in a neighbouring cell.] The graft hybrids pronounce distinctly against the exclusive allotment of the idioplasm to either the nuclei or the chromosomes (compare, DE VRIES, 1903, for another interpretation of graft-hybrids [and NOLL, 1905]), and we may expect interesting lights on the question of heredity from a more exact study of this kind of hybrid.

Bibliography to Lecture XXIX.

BEIJERINCK. 1901. Bot. Ztg. 59, 113.
BOVERI. 1902. Verhdl. d. Gesell. d. Naturforscher. Hamburg, 1901, p. 44.
[BOVERI. 1904. Konstitution d. chromatischen Substanzen. Jena.]
BRAUN, ALEX. 1851. Erscheinung d. Verjüngung i. d. Natur.
BRAUN, ALEX. 1857–9. Ueb. Parthenogenesis. (Abh. Berl. Akad.)
BURCK, W. 1900. Proc. Kon. Akad. v. Wetensch. Amsterdam.
CORRENS. 1899. Ber. d. bot. Gesell. 17, 410.
CORRENS. 1900 a. Ibid. 18, 158.
CORRENS. 1900 b. Bot. Ztg. 58, 229.
CORRENS. 1901 a. Ber. d. bot. Gesell. 19, 71.
CORRENS. 1901 b. Bibliotheca botan. 63.
CORRENS. 1902. Bot. Ztg. 60, 65.
CORRENS. 1903. Ibid. 61, 113.
[CORRENS. 1905. Ueber Vererbungsgesetze. Berlin.]
DARWIN. 1876. Effects of Cross and Self-fertilisation. London.
[DARWIN. 1868. Das Variieren d. Pflanzen u. Tiere. Vol. II. 180. Leipzig.]
[DE VRIES. 1903. Die Mutationstheorie. II. Leipzig.]
[DIGBY. 1905. Proc. Roy. Soc., B. 76, 463.]
DIXON. 1894. Annals of Botany, 8.
ERNST. 1901. Flora, 88, 37.

[Farmer, Moore, Digby. 1903. Proc. Roy. Soc. 71, 453.]
Focke. 1881. Die Pflanzenmischlinge. Berlin.
[Godlewski. 1905. Anzeig. d. Krakauer Akad. 501.]
Guignard. 1891. Annales Sc. nat. VII, 14, 163.
Guignard. 1899. Revue gén. de Bot. 11.
Guignard. 1900. Annales Sc. nat. VIII, 11, 367.
Guignard. 1901. Journal de Bot. 15, 37, 205, 394.
Haberlandt. 1902. Sitzungsber. Wiener Akad., Math.-Nat. Kl. 111, Abt. I.
Häckel. 1866. Generelle Morphologie.
Häcker. 1902. Ueb. d. Schicksal d. elterl. u. grosselterl. Kernanteile. Jena.
Hansen. 1881. Abh. d. Senkenbergschen Gesell. 12.
Hanstein. 1877. Bot. Abhand. 3, Heft 3.
Hegelmaier. 1901. Ber. d. bot. Gesell. 19, 488; ibid. 1903, 21, 6.
Juel. 1900 a. Svenska Akad. Handlingar, 33.
Juel. 1900 b. Jahrb. f. wiss. Bot. 35, 626.
[Juel. 1904. Review in Bot. Centrbl. 95, 361.]
Klebs. 1899. Biol. Centrbl. 19, 209.
Köhne. 1901. Gartenflora, 50, 628.
Kölreuter. 1761–66. Vorl. Nachricht von e. das Geschlecht der Pflanzen betr. Versuchen u. Beobachtungen. Leipzig. (Ostwald's Klassiker, Nr. 41, 1893.)
Körnicke. 1901. Sitzungsber. niederrhein. Gesell.
[Körnicke. 1904. Bot. Ztg. 62, II, 305.]
Loeb. 1899. Amer. Journ. of Physiol. 3, 135.
Loeb. 1902. Archiv f. Entw.-Mechanik, 13, 481.
[Massart. 1902. Bull. Jard. bot. d. Bruxelles.]
Mendel. 1866 and 1870. Ostwald's Klassiker, Nr. 121 (1901).
Meyer, A. 1902. Bot. Ztg. 60, 139.
Miehe. 1901. Flora, 88, 105.
Millardet. 1894. Mém. Soc. sc. phys. et nat. Bordeaux, 4.
Müller-Thurgau. 1898. Landw. Jahrb. d. Schweiz. (Bot. Centrbl. 1899, 77, 135.)
Murbeck. 1901. Lunds Univ. Årsskr. 36.
Nägeli. 1884. Theorie d. Abstammungslehre. Munich and Leipzig.
Nathansohn. 1900 a. Ber. d. bot. Gesell. 18, 99.
Nathansohn. 1900 b. Jahrb. f. wiss. Bot. 35, 48.
Naudin. 1862. Nouv. Rech. sur l'hybridité d. Végét. Paris.
Nawaschin. 1898. Bullet. Acad. St-Pétersbourg.
Noll. 1902. Sitzungsber. niederrhein. Gesell., 10 Nov. 1902 [and 1905 do. do.]
[Němec. 1904. Sitzungsber. Kgl. Gesell. Prag, 13.]
[Ostenfeld. 1904. Ber. d. bot. Gesell. 22, 537.]
Rostafinski, cf. Giard. 1901. C. rend. Soc. Biol.
Solms-Laubach, Graf zu. 1900. Bot. Ztg. 58, II. Abt. 376.
Strasburger. 1878. Ueb. Polyembryonie. Jena. Comp. also Braun (1857-59) and Hanstein (1877).
Strasburger. 1884. N. Unters. über d. Befruchtungsvorgang. Jena.
[Strasburger. 1886. Jahrb. f. wiss. Bot. 17, 52.]
Strasburger. 1900 a. Histol. Beitr. 6. Jena.
Strasburger. 1900 b. Bot. Ztg. 58, 293.
Strasburger. 1901 a. Jahrb. f. wiss. Bot. 36, 493.
Strasburger. 1901 b. Bot. Ztg. 59, 353.
[Strasburger. 1904 a. Sitzungsber. k. preuss. Akad.]
[Strasburger. 1904 b. Jahrb. f. wiss. Bot. 41, 88.]
[Strasburger. 1905 a. Ibid. 42, 1.]
[Strasburger. 1905 b. Die stofflichen Grundlagen d. Vererbung. Jena.]
Treub. 1882. Annales Jard. bot. Buitenzorg, 3, 122.
Tschermak. 1900. Ztschr. landw. Versuchswesen in Oesterreich.
Vöchting. 1892. Die Transplantation. Tübingen.
De Vries. 1889. Intrazellulare Pangenesis.
De Vries. 1900. Ber. d. bot. Gesell. 18, 83.
Wasiliewski. 1903. Jahrb. f. wiss. Bot. 38, 377.
Weismann. 1892 a. Das Keimplasma. Jena.
Weismann. 1892 b. Aufsätze über Vererbung. Jena.
Weismann. 1902. Vortr. über Descendenztheorie. Jena.
Winkler. 1900. Nachr. d. K. Gesell. Göttingen, Math.-Phys. Kl.
Winkler. 1901. Jahrb. f. wiss. Bot. 36, 753.

WINKLER. 1902. Bot. Ztg. 60, II. Abt., 264.
WINKLER. 1903. Ber. d. bot. Gesell. 21, 96.
ZACHARIAS. 1901. Ber. d. bot. Gesell. 19, 377.

LECTURE XXX

VARIATION. ADAPTATION. ORIGIN OF SPECIES

(See note beneath the title of Lecture XXIX.)

IN the last lecture we considered the subject of hybridity with the view of gaining some acquaintance with the significance of fertilization. The facts brought forward introduced, however, another problem of even greater interest, viz. that of the transmission of characters in reproduction. It may be shown that when hybridization is effected there is a hereditary transmission of the peculiarities of *two* organisms, so that the offspring differs in appearance from both parents. Although the variations so induced are often by no means permanent, either because segregation occurs or because many hybrids are sterile and hence produce no offspring at all, there are, on the other hand, hybrids, both natural and artificial, which are completely fertile, and in which the deviations from the parental types are permanent, or, in other words, *new plant forms* may arise by hybridization. Two questions now suggest themselves; in the first place, are the multitudinous forms which we meet with in nature the result of crossing of a few original types? And, again, is crossing the only cause of variation in nature, or, conversely, were it not for this crossing, would the offspring produced sexually or asexually always resemble their parents in all essentials?

If for the term 'types' we read 'species', we have stated the problem, *par excellence*, which has exercised the minds of biologists since the middle of the last century, viz. the problem of the *origin of species*. In the treatment of this problem biologists have collected an overwhelming amount of evidence, zoological, botanical, and palaeontological, all tending to show that the species which now inhabit the surface of the earth have been derived from other different types which previously existed on it. This view has been briefly termed the Theory of Descent, a theory which, as is well known, was brought into prominent notice by the immortal CHARLES DARWIN, who succeeded where previous supporters of the theory were less fortunate. The facts on which the Theory of Descent is based are essentially morphological and systematic, but the experiences of the gardener and the agriculturist have also proved of immense value as corroborative evidence. Plant physiology, however, has in the past been only to a limited extent concerned with this problem, and it is only of recent years that it has been recognized that experimental physiology might aid in the solution of such problems. It is obvious that physiology *alone* is able to shed light on the modifications which species undergo, and to elucidate the more immediate conditions of such changes—to answer the questions *how*? and *why*? Of course experiment cannot tell us how the *present* vegetation came *into existence*—that is an historical question whose solution cannot be reached even in the most elementary form, since the necessary documents are available only to the most meagre extent. The task of physiology is to study the *changes which go on in plants at present living*, to measure their extent, and to investigate their causes. From that study one may draw conclusions as to the phenomena which took place in earlier periods of the earth's history, and so it is possible for physiology and morphology to work hand in hand in this field of inquiry.

Although, even now, physiological data on this subject are extremely limited, still it is of the greatest importance that a brief summary of these should be presented in treating of the physiology of form changes. Owing to the great part which CHARLES DARWIN played in the founding of the Theory of Descent it will be most convenient to start with a very brief consideration of his conception of the origin of species, more especially as his views are now very generally accepted.

DARWIN (1860) compared the *origin of species in nature* to the *origin of races in cultivation*. The formation of different breeds starts with individual variation, that is to say, with the fact that the offspring of the same two parents are not similar in all respects ; of this variation there can be no doubt. The breeder selects for propagation only such organisms as exhibit a certain desired peculiarity, and he expects this character to be transmitted to their offspring in turn. The appearance of variations and their inheritance is entirely a natural phenomenon ; what the breeder does is merely to select (artificial selection) for propagation certain definite individuals. DARWIN believed that a process, similar in all respects to that carried out by the breeder, could be recognized in nature. From each animal or plant so many offspring originate that only a fraction of them are able to find the necessaries of life ; the rest 'succumb in the struggle for existence'. If it be asked how this struggle for existence brings about *natural selection* we believe DARWIN to be correct in his assumption that all the better equipped individuals will have a better chance of remaining in existence and producing progeny than those less well adapted. Whether the organism is well or less well adapted depends on its capacity for making use of all that is favourable in its surroundings and for guarding itself against what is injurious. Let us imagine two seeds of the same species germinating in close proximity to each other ; the one seedling produces its root system rather more rapidly than the other and seizes on the water and nutritive salts in the soil before the other succeeds in doing so. The result will obviously be that the former plant will be successful in the struggle for existence, while the other will be dwarfed. The same result will come about if differences arise in the rate of growth of aerial organs, and a rapidly-growing seedling will deprive the other of the essential light rays. If a seedling contains some poisonous materials which may act as protective agents, and if it possesses some mechanical protective structures, such as raphides or thorns, it will be better protected from the attacks of animals, and have a greater chance of handing on its characters to offspring than the plant that is destitute of such. In nature, as in artificial cultivation, only some individuals reach the propagative stage, and if they transmit their characters then these will be gradually emphasized, and species must alter as they become more and more adapted to their conditions of life.

Considering now the Darwinian theory more in detail, it is obvious that we must give careful attention to the three factors which are specially concerned, viz. variation, heredity, selection. Let us begin with the last, and ask ourselves how it affects species formation. In attempting to answer this question we cannot avoid discussing briefly the significance of the term 'species'. The idea of species is a purely *abstract* one ; *in nature there are no species—only individuals*. By a species we mean the *totality of individuals* which belong to the same line and which preserve their same characters for successive generations. In nature, however, we know nothing of the genealogy of each individual, and regard as a species all those plants which agree in all essential features and live under as nearly as possible similar external conditions. Since it is obvious that different botanists may hold entirely different views as to what constitutes an essential and what a non-essential character, there arise all sorts of discrepancies in the identification and naming of forms. These discrepancies are aggravated according to the varying accuracy of the investigations carried out and the tendency of the investigator to lay emphasis on general characters possessed in common by

the greatest possible number of individuals, or, conversely, the greatest possible differences between individuals. The first type of investigator tends to widen the conception of species, the second to restrict it; LINNAEUS's species may be taken as representative of the former, JORDAN's of the latter. Looked at from LINNAEUS's point of view JORDAN's species are to be regarded as 'petites espèces,' sub-species or as varieties, from JORDAN's point of view LINNAEUS's species are to be considered as collections of species or sub-genera. According to the end the author has in view the limits of the species will sometimes be wide, sometimes narrow. Obviously for our purpose the more restricted the limits of species are the better, for if only their origin can be cleared up, the application of the Theory of Descent to the origin of higher groups (sub-genera, genera, families) presents no essential difficulty whatever.

Let us take as an example the numerous forms of LINNAEUS's species, *Draba* (*Erophila*) *verna*, studied by JORDAN (1873), DE BARY and ROSEN (1889). JORDAN distinguished more than 200 forms, each of which preserved its own special characters for many generations with complete constancy. There can be no doubt that more extended investigations would have resulted in the discovery of an even greater number of forms, distinguished by minuter differences, so that, in short, there would appear to be no limits to species-mongering. How are the individual sub-species of *Erophila verna* distinguished? In addition to the general characters, which are difficult to analyse, there are the differential characteristics of form (contour, margin) more especially of the leaves of the radical rosette, the form and number of the hairs, the appearance of the floral leaves and the fruits. Without further description we may refer to Fig. 113 from one of ROSEN's illustrations.

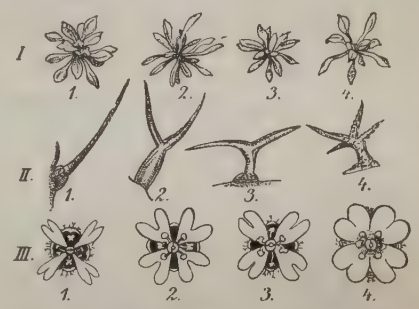


Fig. 113. *Erophila verna*. After ROSEN (1889, pl. 8). *I*, leaf rosette of *Erophila* spec. (*1*), of *E. graminifolia* (*2*), of *E. subtilis* (*3*), of *E. procerula* (*4*). *II*, hairs of *E. oblongata* (*1*), of *E. procerula* (*2*), of *E. graminea* (*3*), of *E. obconica* (*4*). *III*. Flowers of *E. violacea* (*1*), of *E. subnitens* (*2*), of *E. scabra* (*3*), of *E. majuscula* (*4*).

It is very difficult in most cases to attribute a *use* to a specific character, and, again, it is just as difficult to furnish evidence that it can be of no service and *cannot* arise in the struggle for existence. Still it must be admitted, as DARWIN's own investigations and later those of STAHL and HABERLANDT show, that many characters previously believed to be of no service to the organism have distinct functions and hence may have arisen by selection. It is always open to the zealous Darwinian to assert that many a feature that is now of no import may have been of service when it first appeared, for indifferent characters, if non-injurious, may also be transmitted. But we have good grounds for taking exception to the view that the species of *Draba* are all adapted forms which have arisen in the struggle for existence. For instance, we very frequently find a certain habitat of very limited extent occupied by several species forming an association. It is highly improbable that each individual species arose as an adaptation to a certain environment and then that these adapted types should be collected together in a new habitat; but even if this assumption be made, it is very remarkable how three forms, let us say, *a*, *b*, *c*, which have originated in three different places, each in complete harmony with its surroundings, should collect in a new surrounding, and all be equally well adapted to the new conditions, so that no one of them drives out the other. In addition to several species occurring gregariously in one region, *one* and the same species occurs in quite different habitats which have so little in common that they have been unable to affect

these species. In a word, look at it as we will, we are compelled to believe that the specific characters of *Draba verna* cannot have been evolved in the struggle for existence, that they are not adaptive characters, and that they are in themselves useless. Many sub-species in addition to those of *Draba verna* are similarly gregarious, others, however, such as those studied by WETTSTEIN, exclude their near relations from their habitats (WETTSTEIN, 1898, Grundzüge d. geographisch-morphologischen Methode der Pflanzensystematik. Jena). What we have said as to sub-species applies to species also of other plants. In all large genera we find species which live in the same habitats, but whose specific characters are in no ways adaptive. Examine the differences, for instance, which exist between *Avena elatior* and *A. pubescens* which grow beside each other in the same field; it is impossible to regard these as adaptive characters; still less can we regard as adaptive the characters which distinguish genera, families, and higher groups. We are inclined to hold that in most cases (p. 395) where two forms are distinguished *only* by dissimilar *adaptive* characters, either we are not dealing with genuine *species* or that their *real* differences have not yet been discovered. In the former case we should be dealing with modifications due to *habitat* only, such as we so often meet with in water and land, light and shade, mountain and lowland forms; they differ from genuine species in this, that their characters are not hereditary, but disappear again when, or soon after, the inducing factors are removed.

At the same time we do not mean to affirm that adaptive characters cannot be inherited. We shall return to that point later on, but meanwhile it may be noted that there are obviously two quite distinct kinds of characters possessed by species, *adaptive* characters and *organic* characters, a distinction which has been emphasized with special clearness by NÄGELI (1884). This distinction may be made out at each stage in the classificatory system. There are aquatic and terrestrial Algae just as there are aquatic and terrestrial Phanerogams, and both Angiosperms and ferns include both xerophytic and hygrophytic types. In smaller groups also adaptive and organic characters may be distinguished. The problem as to the origin of species is primarily the problem as to the origin of definite *organic* characters; however, since all species exhibit adaptive characters as well, we must study their origin also.

From what has been already said it must be admitted that DARWIN'S principle of natural selection cannot explain the *origin of species*. Let us see whether it is sufficient to explain *adaptive* characters. Here again, however, we meet with great difficulties. According to DARWIN, the differences between competing individuals are not great but they become gradually intensified by summation in the course of an indefinite number of generations. Consider, for example, some peculiarity of a plant obviously of service to it, such as the prickles of a rose which aid it in climbing, or the spines of a thistle which protect it from the attacks of animals; according to DARWIN'S theory these prickles and spines began as excrescences of minimum height on a previously smooth plant and attained their present structure and dimensions gradually. Only after the organ had become sufficiently prominent, however, could it have been of any use to the plant; in a word, it is easy to understand how DARWIN'S theory may explain the *improvement* of an organ already in *existence*, but it does not make clear how it first arose.

Having seen that the mode of operation of natural selection as defined by DARWIN does not afford a satisfactory explanation of adaptive, let alone specific, characters, let us next inquire as to DARWIN'S interpretation of variation and of heredity. It will be necessary to study these two questions in conjunction, since the important point for consideration is *whether or not variations are inherited*. DARWIN assumed that *every* new character, however it arose, was capable of being inherited, but this assumption has yet to be proved, and especially in reference to each type of variation, of which we may distinguish

three (DE VRIES, 1901 a) (compare also KLEBS, 1903, as to the types of variations and as to the origin of species), fluctuating variations, adaptive variations, and mutations). To these may be added a fourth type, variations which arise from hybridization ; into these, however, we cannot enter here.

Fluctuating variations may be termed also *individual*, because they show themselves in single members of the species, arising apart altogether from crossing or extraneous influences of that sort. If the seeds from a single capitulum of a member of the Compositae be planted in a garden the plants which result vary extremely in weight and size, and, later, the different organs of each plant also vary in number, size, and weight from the quantitative aspect. If statistics be collected of a larger number of individuals a certain average may be determined for each character, and the deviations from that mean occur less and less frequently the greater they are. We may, in fact, from the data so established construct a curve (Galtonian curve) which corresponds more or less exactly to the law of probabilities (QUETELET). The curve has one crest decreasing rapidly to zero on either side. These individual variations may be illustrated first by a few examples taken from observations on wild plants selected at random.

Number of rays in the archegoniophore of *Marchantia* (LUDWIG, 1900, p. 22) :—

No.	7	8	9	10	11	12	13	
Frequency	1	14	307	152	44	3	1	Total 522.

Number of petals in *Linaria spuria* (VÖCHTING, 1898) :—

No.	2	3	4	5	6	7	8	9	
Frequency in actinomorphic flowers	1	2	43	810	52	2	1	1	Total 912.
„ „ zygomorphic „	—	4	240	60250	169	7	1	—	Total 60671.

Length of seeds of *Phaseolus vulgaris* (DE VRIES, 1901 a, p. 34):—

Mm.	8	9	10	11	12	13	14	15	16
Frequency	1	2	23	108	167	106	33	7	1

Percentage of sugar in 40,000 beets from Naarden (DE VRIES, 1901 a, p. 74):—

% sugar	12	12½	13	13½	14	14½	15	15½
Frequency	340	635	1192	2205	3597	5561	7178	7829
% sugar	16	16½	17	17½	18	18½	19	
Frequency	6925	4458	2233	692	133	14	5	

The data given in this last example are also expressed in the form of a curve in Fig. 114. It should be noted that curves with several maxima occur, and also half curves as well, but these cannot be further discussed.

We have now to discuss what is the relationship of these individual variations to species formation. First of all, we must note that there is a very close connexion between such variations and the mode of formation of agricultural breeds or races by selection. Thus, in the sugar-beet industry, the selection and employment for propagation of the seeds of those plants which are richest in sugar leads to a marked rise in the general average of the sugar percentage in the beet. Fifty years ago the percentage was 7–8 per cent., now it has been raised to about 15 per cent. In the same way, by rigorous selection, races may be produced which will exhibit especially large flowers or fruits, better flavour, increased succulence, &c. So far as we know, no new characters arise spontaneously, although those already existent may be added to or reduced. The limits of such variations are usually reached in a few generations (3–5) ; further selection merely serves to fix the character which has been acquired. It must be specially noted, however, that such characters are liable to fade away quite as rapidly as they appear ; after a few generations, if selection be discontinued, the original condition is reverted to. Herein lies a great distinction

between artificially produced races and natural species : the former are transitory, the latter are constant.

It has been said above that the most important point about a variation is whether it is hereditary or not. Are these individual variations hereditary or not ? This is a question by no means easy to answer. The beetroots, from which the graphic curve given at Fig. 114 was taken, arose from seed, which had been obtained from plants possessing 16–18 per cent. of sugar, and yet by far the majority of them have less than 16 per cent., and individuals themselves differ very greatly. We have to deal here only with a ' partial ' inheritance, since only *some* of the characters of the parents reappear in the offspring. On the whole, this is probably not a case of heredity. Our position in relation to this question will be more secure after we have successfully determined the *factors* which induce individual variations. Meanwhile, we may suggest a hypothesis which may be considered as highly probable. Fluctuating variations must arise from *irregularities* in growth conditions (nutrition in the widest sense of the word) which must arise even in the most carefully conducted experiments. [Compare KLEBS, 1903.] Indeed, one cannot prevent one plant from taking up more water or mineral constituents from the soil or absorbing more light than another ; similar organs on the same axis must be differently nourished according to their position, for it may be readily proved that the flow of nutritive materials to a terminal organ cannot be equal to that which reaches a similar organ in a lateral position. The lateral inflorescences of the sunflower, for instance, become considerably larger when the terminal inflorescences are removed, and many examples of like character might be quoted in support of this view. When one flower is better nourished than another larger seeds will naturally result, as also larger embryos and more abundant reserves. Again, a plant which has arisen from a large seed will in turn tend to grow more vigorously, produce a greater number of seeds, and develop greater quantities of sugar. If individual variations be really due to nutritive influences *only*, we cannot speak of an inheritance of such peculiarities in a single individual, and, at the same time, readily account for the rapid disappearance of these characters when selection is discontinued. Further, it is conceivable that selection may be aided or even replaced by good manuring. [As the result of JOHANNSEN'S (1903) researches it is clear that even races which breed quite true are always the result of the mingling of several forms differing from each other in minute but constant characters. If we start from the offspring of a self-fertilized plant such offspring also exhibit differences among themselves, which, when plotted out, give a graphic curve such as that shown in Fig. 114. If from among such forms it be desired to produce a breed with a certain definite character, and if we select for that purpose seeds for a third generation from plants standing at the very beginning or the very end of the curve, it will be found that the *two* sets of plants resulting present no differences ; in fact, selection, in this case also, is quite useless. If selection appears to have a different effect in the other experiments already spoken of, it must be assumed that we are dealing in these cases not with individual variants but with mutation phenomena. Compare also CORRENS, 1904.]

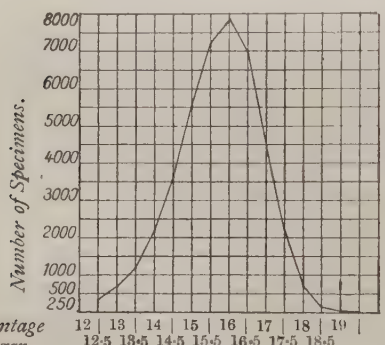


Fig. 114. Percentage of sugar in 40,000 beets, expressed graphically. DE VRIES (Mutationstheorie, I).

Just as the first type of variations may be referred to the influence of external factors, so also the second type, viz. adaptive variations, may be regarded as closely related [DETTO, 1904]. By adaptive characters we understand variations, many of which (p. 391, under 4) are induced by external factors, changes which are characterized by some quite special feature, and which may be considered as a *purposeful* reaction. Examples of such reactions are to be met with everywhere, and many instances have already been given of these in Lectures XXIV and XXV. The external factor in these cases acts as a stimulus, and the reaction consists in a morphological or anatomical alteration, calculated to render the plant more capable of making the best use possible of its surroundings, or of protecting itself from injurious influences in its environment—as we may term the *sum total* of the external factors which affect it. The *capacity* for so adapting themselves is possessed by different plants in very varying degree, and hence some are able to thrive in the most varied situations, whilst others are injured; in some cases fatally, by extremely slight deviations from optimal conditions. Even the most adaptive of plants have, however, their limitations, for although amphibious plants can live in water as well as on land, there is usually in the long run a certain minimum and a certain maximum degree of dampness which may not be exceeded; in other words, amphibious plants cannot on the one hand become aquatics nor on the other xerophytes. According to MASSART (1902), however, *Polygonum amphibium* may assume a xerophilous form and structure. It is impossible to say how this widely distributed capacity to react adaptively to external stimuli has arisen, but it must be assumed that external influences have been operating on the plant world in the same way and in no greater variety, for thousands of years, and that, in the struggle for existence, those plants which failed to react by adapting themselves in this manner succumbed. In other words, the external stimuli at first resulted in reactions, some of which were adaptive and some not, and by natural selection only those which responded in a suitable manner remained in existence ; the offspring of these plants would inherit such peculiarities and so gradually the *power of adaptation would become fixed by heredity*. These are hypotheses, however, into which we need not go any further. But we must emphasize the fact that by no means *all* stimuli induce purposeful reactions. The gall, for example, is of service only to the insect, but is highly disadvantageous to the plant ; we must assume indeed, by way of explanation, that the insect succeeded in deluding the plant, so that instead of treating the insect as an enemy and an intruder it behaved towards it as if it were a bit of itself. Under other conditions, also, we meet with non-purposeful reactions, such as those which result from the application of an unwonted stimulus to which the plant is not subjected in a state of nature, and to which it has had no opportunity of adapting itself. We know of no cases of *alteration in form* which would serve as examples of such reactions, but illustrations frequently occur, especially in the phenomena of movement, as when a bacterium is attracted by ether, which is of no service to it, and is not repelled by corrosive sublimate, which is fatal to it ; or when a root bends towards light, and a tendril refuses to curve round a stick smeared with gelatine. We may well believe—to select the last case—that the tendril would grasp such a support if it had often the opportunity in nature of meeting with supports possessing a gelatinous surface.

As to the causes inducing this adaptive capacity perhaps the best sources of information are studies on unusual and artificial stimuli, especially as they have been systematically investigated, and, further, since it is very doubtful whether it is possible to study a ' natural ' stimulus of any kind which the plant is not already acquainted with. GOEBEL (1898) has drawn attention to the response given by *Cardamine pratensis*, which, according to SCHENCK (1884), can produce a typically aquatic form, although it usually occurs on land ; but

in the case of meadow plants, which are not infrequently subject to inundation, the special adaptation to aquatic life may have arisen a long time before the capacity for adaptation had come to be inherited.

Hitherto we have dealt with the so-called ' active ' adaptations only, but there are also other adaptations which may be termed '*passive*'. There are not only plants which are *able to adapt themselves*, but also others which *are adapted*, which exhibit a series of peculiarities which permit them to live under certain environmental extremes. In addition to the genuine aquatics we have also hygrophilous types (such as the Hymenophyllaceae), the numerous xerophytes, and halophytes (or salt plants), and a general consideration of such passive adaptations leads us to the conclusion that these have arisen by a hereditary fixing of active adaptations. In many liverworts we find that the shape of the thallus is dependent on light, for it remains narrow in light of limited intensity and broadens as the degree of illumination increases, thus exposing a maximum surface at right angles to the incident ray. The flattened form of the green assimilatory organ has, as we know, a definite purpose. In the lower plants this is a case of active adaptation, whilst in the leaf-blades of the higher plants the adaptation has become fixed by heredity. The same is true of the roots of many epiphytic orchids, where in many species these organs become flattened when exposed to light, although in other cases the flattening is always present, even when the roots are grown in the dark (GOEBEL, 1898). Numerous examples of the same kind might be quoted, especially in relation to the phenomena of dorsiventrality and polarity, phenomena which—although, perhaps, they should not, strictly speaking, be classed among adaptations—may be referred to here because they can in many cases be readily shown to be due to external factors such as light and gravity, although in other cases they have arisen without such stimuli. Thus VÖCHTING (1886) showed that the flowers of *Epilobium angustifolium* and of *Hemerocallis fulva* owe their dorsiventrality to gravity and that they become radial when this unilateral stimulus is withdrawn. Dorsiventrality appears in *Amaryllis formosissima* under all conditions, however, and gravity merely renders the dorsiventrality more intense.

As has been already said, a general comparison of examples compels us to believe in the derivation of the passive from the active adaptations, hence these active adaptations must be capable of transmission, and yet experiment does not confirm this conclusion. Plants which have lived in the high Alps for thousands of years, and which have adapted themselves to their surroundings by taking on very characteristic forms, lose all these peculiarities when they are cultivated in the plains below. Conversely, lowland plants transplanted to an alpine habitat take on an alpine form but lose the adaptations which they thus acquire when once more brought back to their original home (BONNIER, 1895). In the same way, in cases where adaptations have been induced experimentally, it is found that these are in no sense permanent, and that the seeds of plants which have been cultivated for a long time under exactly similar external conditions still retain complete power of adapting themselves. The gap between practice and theory can at present be bridged by hypotheses only. Perhaps an active adaptation induces a certain effect on the idioplasm, so that it disposes it to repeat more readily this adaptation than any other. The initiation and disappearance of adaptations take place often not in *one* generation but in the course of several; the influences which have been operative in the first generation obviously have still some effect in the second, and, if that after-effect be combined with the new influences, at first there will be only a partial inheritance, and a complete transmission only after several generations. We are also acquainted with changes in the plant which outlast the stimuli which induced them, and we have, in our discussion of periodicity, recognized *after-effects*, which may be compared to a certain extent with the partial transmission postulated above.

But we can scarcely rest satisfied with this hypothesis only; we must also assume a *definite capacity in the plant of establishing certain adaptations by heredity*, and this capacity is certainly not universally possessed. To this view, however, scarcely any exception can be taken, since it is a well-known fact that the capacity for adaptation is especially pronounced in some circles of relationship whilst it is absent from others. This is shown very strikingly by the way in which some families among higher plants tend to a parasitic or carnivorous habit while others exhibit no such leaning. Similarly, a tendency to inherit an adaptation to an aquatic or to a xerophytic life may exist here and there, whilst other plants, perhaps, have, on the contrary, lost that adaptive *capacity*. Two principles, in a certain degree antagonistic, make themselves evident here, the one aiming at making the plant as many-sided as possible and so permitting it to find a footing in *numerous* different situations, the other making it one-sided but also gifting it with the capacity of adapting itself to one *extreme* condition. If all organisms made exactly the same demands on the environment their continued existence would be a mere matter of days. The correctness of this conclusion has already been emphasized elsewhere (Metabiosis, Lecture XIX).

Weighty objections to the inheritance of acquired characters have been advanced in the animal world, especially by WEISMANN. He (1892) regards adaptations as acquired characters, and tries to show that inheritance of these is theoretically impossible and has never been established practically. This view of acquired characters is, in the first instance, based on a study of the animal world, where there is frequently a sharply marked demarcation between germ and somatic cells from the very commencement of the divisions in the egg-cell. The peculiarities which occur in the somatic regions induced by external influences or functional stimuli are regarded as acquired, or originating during the life of the individual, in contrast to those which are inherent, that is, whose initials were already present in the ovum. We may distinguish four types of acquired characters: (1) mutilations; (2) diseases; (3) adaptations to external conditions; (4) functional adaptations. Botanists and zoologists are agreed as to the non-inheritance of changes which are induced by mutilation and disease. That there is *no direct evidence* of the inheritance of adaptations due to external factors, was admitted above. The same is true of functional adaptations; VÖCHTING (1899) has made many experiments on the subject and has established beyond all question that such adaptations are not hereditary. The question comes to be, whether any *fundamental* considerations can be advanced *against* the assumption of their transmissibility. That would be the case if we conceived of the origin of the adaptations in the individual as the zoologists often appear to do; for they assume that any alterations in the somatic-cells must be appreciated by the germ-cells, but that can scarcely occur unless by a transference of idioplasm from the somatic to the germ-cells. Such an assumption (Pangenesis, DARWIN, 1868) verges too near to empiricism, and it would appear to us that such an idea is not only unessential for the explanation of the phenomena as presented by the vegetable kingdom, but is in itself quite *incorrect*. Let us study a single example of adaptation in the plant. If we place a land plant in water we *do not* find that leaves already present change their shape and structure, but die off just because they no longer possess the power of adapting themselves to their new surroundings; on the other hand, adaptations appear in the quite embryonic leaf-initials, close to the growing point, where germ-plasm or idioplasm is much more abundant than in the full-grown parts. We find, that is to say (and this is of general significance), that the adaptation does not take place in the soma proper, but in the growing point. It is from the growing point, however, that the reproductive cells are also derived, and they are able to receive adaptive impressions without the inexplicable transference of a material basis from the soma. Certainly we must assume the transference of a stimulus, inas-

much as the growing point is not itself in contact with the water and can only be indirectly effected by it (p. 339). Similarly, in other cases, as when leaves take on forms adapted to light or to shade, the relationship of light to the growing point enclosed in its scales must be the same in both cases, although a quite different type of leaf is differentiated in the bud of the shaded branch from that of the illuminated one. Experimental investigations on this subject are, however, much required. As NORDHAUSEN (1903) has shown, the characteristic anatomical differences between light- and shade-leaves of the beech are already established in the bud, and the light relations concerned in the unfolding of the bud play only a limited part in the process. If we arrange that the initials of the light-leaf are allowed to develop in the dark, the typical double row of palisade-cells (Fig. 115, *I*) is retained, whilst the shade-leaf retains its own characters, although it be developed in bright sunlight (Fig. 115, *II*). The *growing point*, in this case, undergoes adaptation, and the effect outlasts the stimulus, so that one may readily conceive how hereditary races may in this way come into existence. As a matter of fact, this is not the case in the *beech* ; further research is needed to show how far the after-effect is continued, whether the branches which have been exposed to light for ten years form leaves of the light-type, after shading, longer than those which have been exposed to intense light for one year.

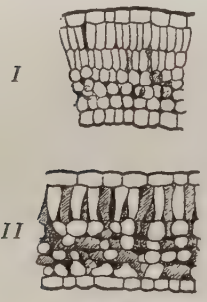


Fig. 115. Transverse sections through leaves of a copper beech, *I*, primordium of a light-leaf developed in the dark, *II*, of a shade-leaf developed in a bright light. After NORDHAUSEN (1903).

Let us ask finally how functional adaptations behave in regard to inheritance. These naturally cannot be clearly demarcated from those we have just spoken of, and so our knowledge with regard to them is even more limited. The functional adaptations with which we are acquainted are, for the most part, the result of long past influences, and we may well assume that they also first made themselves apparent at the growing point. Where that is not the case, as in VÖCHTING'S experiments (stem tubers of *Boussingaultia*, leaf tubers of *Oxalis*), no transmission can be determined. WINKLER (1902) has recently drawn attention to a case of great interest in this connexion. He observed floral-leaves and styles of a chrysanthemum become green after flowering was over, and exhibit all sorts of anatomical alterations, which were functional adaptations permitting assimilation to be carried out. We do not know whether these malformations were transmissible and we are ignorant as to their causes, nor can we assert that they were first operative in the full-grown organ.

The ideas which we have formed as to the inheritances of acquired characters may be more briefly expressed. We may say that *there are no* acquired characters in the sense indicated above. The characters do not appear in the soma generally (GÖTTE, 1898) but at the growing point, and so far the possibility of their inheritance is granted. In lower organisms, which, as a rule, show no differentiation of somatic and germ plasma, it is quite obvious how the effect of external influences may be inherited (and several observations have been made on the subject), but perhaps no special adaptations may occur in such cases, since the effects produced are of no service to the organism (for literature, see PFEFFER, Phys. II, 242). We have succeeded, by special means, in developing certain races of Bacteria which have permanently lost the power of producing colouring bodies or certain special poisons. We have also been successful, by prolonged culture at high temperatures, in destroying the power of forming spores in various Saccharomycetes. The point which is characteristic in this process is the *gradual* fixation of the loss ; at a definite high temperature the formation of spores ceases, but the capacity for forming them returns when the temperature is

again reduced, and it is only after long-continued exposure to high temperatures that a constantly asporogenous race is formed.

Let us now turn to the third type of variation, viz. mutations. These have come into special prominence of late years owing to the labours of DE VRIES (1901) and KORSCHINSKY (1901). By mutations we understand variations which, once initiated, are thereafter quite constant. Mutations are variations which appear *spasmodically*, one or more characters of a species suddenly exhibit alteration or appear *ab initio*, and these may show themselves for the first time in a plant which has arisen from a seed or a single bud. Let us consider first of all a few examples of malformations which appear to be of the nature of mutations. Look at the plants which possess branched leaves. The oldest example is *Chelidonium laciniatum*, a form of *Chelidonium majus* which suddenly made its appearance at Heidelberg in 1590, a form possessed of branched leaves, both floral and vegetative, and which has constantly reproduced these characters by seed from that day to this. The double flowers which occur in many plants have arisen by mutations, as also cases of fasciation, best known in the commonly cultivated plant *Celosia cristata*. Further, very characteristic examples are seen in the rayless Compositae e.g. *Matricaria discoidea*, the thornless varieties of plants which usually possess thorns, e.g. on the fruit (*Ranunculus arvensis inermis*, *Datura tatula*, &c.). Again, we have the notable case of *Capsella heegeri*, which was found growing wild near Landau, and which has been shown (SOLMS-LAUBACH, 1900) to be a mutation of *Capsella bursa-pastoris*. As an example of bud variation equally well known we may select the case of *Sedum reflexum*, recorded by WETTSTEIN (1900) as having been found near Prag, which showed fasciation on a single lateral branch. This branch, when cultivated, flowered, and from the seed arose again magnificently fasciated examples.

The reason why mutations are of so much importance for the theory of the origin of species is especially because, in addition to the constancy of the new character, the innovation is in no sense an adaptation but entirely a mark of *organization*. The mutations cited, however, differ certainly in one characteristic only from the parent species, such as we find in those natural forms termed by many authors *varieties* (e.g. white-flowered varieties), but not in 'elementary' or sub-species, which usually exhibit differences in *all* characters. The direct observation of a new sub-species by mutation is to be considered as a great advance in our knowledge, and this observation we owe to DE VRIES (1901 a). He cultivated many specimens of *Oenothera lamarckiana*, an American immigrant into Europe, and now partly naturalized there, and was able to establish the occurrence of many mutations which differed from the type in many or in all characters. The offspring arising from one seed were constant in exhibiting the new character or characters. We shall limit ourselves here to the consideration of one example only, viz. *Oenothera gigas*. Let us hear what DE VRIES (1901 b) has to say on the subject:—'It is of the same height as the parent species, but the stem is thicker, the leaves are more numerous, the corolla is more widely opened, and the buds are much thicker (compare Figs. 116 and 117), the fruits are only half as long as the fruits of the parent, and they contain fewer seeds. The seeds are larger, rounder, and heavier. This type arose in my garden in the year 1895 as a solitary case amongst 14,000.' By preventing any crossing, pure seeds of this type were gathered in 1896. 'These were planted in 1897. *As soon as the third and fourth leaves had unfolded, the differences appeared. All the young seedlings were stronger and more fully clothed with leaves and darker in colour than the parent.* There were several hundred such, but obviously all of one type only, and as in the course of the summer, first the stem and then in succession the leaves, the buds, the flowers, and, finally, the fruits showed themselves, all doubt was removed that a new and constant species had made its appearance.

Taking origin from a solitary example, *O. gigas* forthwith appears with constant and pure seed; arising at a bound from the parent form, in a moment a new species sprung into independent existence. In this way also the other species I have described arose, suddenly and without any transitional forms.'

There are a whole series of questions which are closely connected with this observation of De Vries, and of these we can touch on only a few of the more important. First of all, how can a sub-species arising from a *single* specimen maintain itself in nature? If we assume that crossing with the type takes place, the species would soon disappear, unless its characters were absolutely predominant; further, no subsequent segregation is possible, as in the case of the hybrid peas quoted previously. That something of this kind may happen is shown by the experiences of the American farmer with the Ancon sheep. There was born

Fig. 116. *Oenothera lamarckiana*. Apex of a shoot at the commencement of flowering; *b*, withered flower lying on the subtended leaf. After De Vries (1901).

Fig. 117. *Oenothera gigas*; derived from *O. lamarckiana* in 1895. Apex of the shoot at the commencement of flowering; at *a*, a petal has been removed; *b*, withering flower. After De Vries (1901).

in Massachusetts in 1891 a lamb with the form of a dachshund. In spite of cross-breeding with the parental stock the Ancon type still predominated, and the race increased without any selection at all, artificial or natural (Darwin, 1868). If we assume self-fertilization, a maintenance of the new species can only take place if it be more dominant than the parent species, and gradually drives it out. The gregarious occurrence of closely-related species would, under these conditions, be difficult to explain. Such being the case, another possibility must be admitted. De Vries observed, in the case of the new species of *Oenothera*, that they arose not once but several times from the parent stock. It seems probable that the old species produced the new ones in ever-increasing numbers. We certainly cannot advance any proof of this assumption, but it may be supposed that the originating of a mutation induced besides an *internal*

alteration in the mutating organism. The new characters must, as NÄGELI (1884) acutely pointed out, be firmly fixed and ready as initials which are at first latent, and later on unfold themselves. When an initial *once* finds the way to complete development then it may go on elaborating in ever-increasing degree. In this way also certain *new* characters would behave just like certain ancestral characters which make their appearance as so-called '*reversions*' whenever opportunity offers, and which generally are transmitted in the *latent* form. We know as little about the causes which lead to their appearance as we do of the origin of mutations. All we can say is that the causes appear to be *internal*, which is equivalent to saying that we know nothing about them.

If then, as DE VRIES thinks, the *mutation*, and not the individual variation, is the factor concerned in the origin of new species, then selection must have a significance quite different from that attributed to it by DARWIN. According to DARWIN, individuals are for ever engaged in a struggle for existence, and new species *arise* by selection of the varieties best equipped for the struggle. According to DE VRIES, however, fully developed species come into conflict, but the origin of these species by mutation is not thereby explained. We have certainly, if we accept DE VRIES'S view, a 'Mutationstheorie' but we have absolutely no *theory of mutation* itself.

The formation of species farther apart out of the fundamental species is, according to DE VRIES, easily understood, since many 'petites espèces' disappear in the struggle for existence. Of course, the mutation may be so great that a new genus or a new family may come into existence. *Capsella heegeri*, for example, would scarcely have been placed in the genus *Capsella* were it not that its origin was known. It is also quite possible that whole genera and families may be referred back in their chief features to monstrosities. HILDEBRAND (1899) has found *Fuchsia* to produce zygomorphic flowers arising by mutation (Fig. 118), so that the allied genus *Lopezia* may have arisen from a malformation (Compare SACHS, 1893).

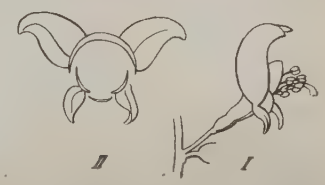


Fig. 118. Zygomorphic flowers of *Fuchsia*. *I*, lateral view. *II*, ground plan. After HILDEBRAND (1899).

HOFMEISTER long ago (1868, p. 564) ascribed to mutation a very prominent part in the formation of species. He said: 'New forms do not come into existence by the summation in successive generations of small differences from the customary form, all tending in the same direction; *they appear suddenly, and are widely different from the parent type.*' It may well be that, as our knowledge increases, the distinction between the different types of variation may be broken down; still it is certain that mutability will remain the *chief* if not the only factor in species formation. That species may also owe their origin to individual variation, in the extreme way suggested by WALLACE, appears to us practically out of the question, but that they may rise by hybridization is, in individual cases, quite established, and that they may owe their origin to adaptation is especially true of those species (biological species) recently recognized among parasitic Fungi. Unfortunately, space will not permit of our discussing these forms. [Compare KLEBAHN, 1904, and DETTO, 1904.]

In this lecture we have not been able to give more than the briefest sketch of the theory of the origin of species, and have been compelled to omit consideration of very many most important observations and views. We must, therefore, refer to the special literature on the subject, in which, it is true, some one 'theory' frequently predominates. The task of the future is less to establish new theories than carry out systematic observations and experiments. General summaries of the Theory of Descent will be found in the following works:—WALLACE, 1891;

Weismann, 1902; Reinke, 1901; Wettstein, 1903; [Plate, 1903; Errera, 1904; Lotsy, 1906].

Bibliography to Lecture XXX.

Bonnier. 1895. Annales Sc. nat. VII, 20, 217.
[Correns. 1904. Archiv f. Rassen- u. Gesell-Biologie, 1, 2.]
Darwin. 1860. Die Entstehung der Arten (German ed. by Bronn from the 2nd Engl. ed.)
Darwin. 1868. Das Variieren d. Tiere u. Pflanzen. German ed. by Carus.
[Detto. 1904. D. Theorie d. direkten Anpassung. Jena.]
[Errera. 1904. Une leçon élém. s. l. Darwinisme. Bruxelles.]
Goebel. 1898. Studium u. Auffassung der Anpassungserscheinungen bei Pflanzen. Munich.
Götte. 1898. Vererbung u. Anpassung. Rectorial Address, Strassburg.
Hildebrand. 1899. Bot. Centrbl. 77, 177.
Hofmeister. 1868. Allgem. Morphologie d. Gewächse. Leipzig.
[Johannsen. 1903. Erblichkeit in Populationen. Jena.]
Jordan. 1873. Rem. sur le fait de l'existence en société à l'état sauvage des espèces végétales affines. Lyon.
[Klebahn. 1904. Die wirtwechselnden Rostpilze. Berlin.]
Klebs. 1903. Willkürliche Entwicklungsänderungen. Jena.
Korschinsky. 1901. Flora, 89, 240.
[Lotsy. 1906. Vorles. über Deszendenztheorien. I. Jena.]
Ludwig. 1900. Bot. Centrbl., Beihefte, 9, 89.
[Massart. 1902. Bull. d. Jardin botan. d. Bruxelles.]
Nägeli. 1884. Theorie der Abstammungslehre. Munich and Leipzig.
Nordhausen. 1903. Ber. d. bot. Gesell. 21, 30.
[Plate. 1903. Ueber die Bedeutung des Darwinschen Selectionsprinzips.]
Reinke. 1901. Einleitung in die theoretische Biologie. Berlin.
Rosen. 1889. Bot. Ztg. 47, 565.
Sachs. 1893. Flora, 77, 234. Note.
Schenck. 1884. Ber. d. bot. Gesell. 2, 479.
Solms-Laubach. 1900. Bot. Ztg. 58, 167.
Vöchting. 1886. Jahrb. f. wiss. Bot. 17, 297.
Vöchting. 1898. Ibid. 31, 391.
Vöchting. 1899. Ibid. 34, 1.
De Vries. 1901 a. Die Mutationstheorie, 1. Leipzig.
De Vries. 1901 b. Die Mutationen u. die Mutationsperioden. Leipzig.
Wallace. 1891. Der Darwinismus. German ed. by Braun. Braunschweig.
Weismann. 1892. Ges. Aufsätze über Vererbung. Jena.
Weismann. 1902. Vorträge über Deszendenztheorie. Jena.
Wettstein, R. v. 1900. Ber. d. bot. Gesell. 18, (184).
Wettstein, R. v. 1904. Der Neu-Lamarckismus. Jena.
Winkler, H. 1902. Ber. d. bot. Gesell. 20, 494.

PART III

TRANSFORMATION OF ENERGY

LECTURE XXXI

FORMS OF ENERGY IN THE PLANT

Side by side with the law of conservation of matter, one aspect of which we have learned to recognize in the circulation of elements in the organic world, we place the law of conservation of energy. According to this law the sum of the energies of the universe is a constant quantity; energy cannot be created or destroyed, it can only be transformed. Thus, for example, heat energy can be transformed into mechanical energy, or electrical energy can be transformed into radiant energy. No special evidence need be adduced in proof of the fact that the laws of conservation of matter and of energy are applicable alike to the living and to the non-living world. The task before us now is to follow the transformation of energy, just as, in Part I, we studied the transformation of matter; that is to say, we must attempt to answer this fundamental question, viz. 'Whence does the plant obtain its energy, and what does it do with it?' Of course, this is not the first time we have encountered this problem, for in speaking of metabolism it was impossible to avoid mentioning the question of the transformation of energy which is inseparable from it. Separation of the treatment of the subject of transformation of matter from that of transformation of energy is merely a matter of practical convenience; in nature they are bound together in the most intimate manner.

Thus we had to draw attention to the fact that light was essential to the assimilation of carbon in the green plant, or, more exactly, that light energy becomes transformed into chemical energy, which latter reappears as potential energy in the products of assimilation. It has also been noted that sunlight is the most important source of energy for all living things, since those plants and animals which are unable to make use of sunlight directly with the aid of chlorophyll, are compelled to absorb the products of assimilation of green plants, and thus acquire the energy of sunlight *indirectly*. Further, it has been already shown that not only light energy but also, in many cases, chemical energy, obtained by the absorption of materials (nutriment), is a primary source of energy in the plant. Nutritive substances are employed, as we have already seen, only to a small extent in the actual manufacture of the organism; the greater part is again broken down in the course of respiration or in other related processes, and thus the chemical energy released in the decomposition of more complex compounds is turned to advantage in the general vital phenomena of plant life. In the process of nutrition, however, the plant acquires other forms of energy as well, which operate quite independently of the energy evolved in metabolism. Thus electric energy, osmotic and surface-tension energy or energy of crystallization of the included substances must be recognized, and these, with the exception of electricity, play, as every one knows, important parts in the plant economy. It is not at all likely that the plant is able to make any use of electrical or mechanical energy, absorbed as such from the environment and not associated with actual materials, at least we are quite ignorant of such cases; on the contrary, we know that the plant thrives perfectly well without

such supplies of energy. It is quite otherwise with heat. It has already been shown that all plant activity is conditioned by certain definite limits of temperature, but it does not follow that the heat of the external medium forms a source of energy to the plant. There can be no doubt that the plant *does* absorb heat from the exterior in cases where it exhibits a lower temperature than the outer world, a condition of things by no means rare ; and if the plant absorbs heat under such conditions obviously its energy must be increased. We need not dwell on this fact, for it is self-evident ; what we must inquire into is whether the plant *requires* such an addition of heat energy from without, but to this question we can give no conclusive answer ; the probability is that it does not require it.

Probably, all the energy required by the plant is obtained in the form of light and nutriment. The law of causality compels us to believe that all the energy of the plant has entered it *from without*, for we cannot conceive of the plant, any more than of non-living matter, creating energy within its own body, so that what we have to investigate is the way in which the energy which enters the plant becomes altered within it. Just as chemical compounds occur in the organism which are manufactured in it only, so there may exist in it forms of energy which occur nowhere else. Such specifically organic forms of energy are, however, unknown. It must be confessed, however, that, we know nothing of energies peculiar to the organism. Even as to the changes which occur in the energy entering the plant we know but little. Since only the final stages in the process which manifest themselves externally are accessible for investigation, we are confined to guesswork as to the transformations taking place within. Among the final stages visible to us the most important is mechanical energy. The movements which the organism, in whole or in part, exhibits are obviously those most prominent and hence also most studied. The production of heat must also be noted as a phenomenon of very wide occurrence, as to the meaning of which, however, we know very little, though we are more conversant with its causes. In addition to heat, the production of electric currents and of light in plants must be referred to, phenomena which, as yet, can only be said to play a subordinate part in plant physiology.

This third section of our lectures must deal almost exclusively with the phenomena of movement, but before we commence their study we may briefly refer to the other manifestations of energy met with in the plant, viz. heat, light, and electricity.

The temperature of the plant conforms, generally speaking, with that of the exterior medium, sometimes it gives off heat to the environment, sometimes it absorbs heat from it ; it is destitute of those special contrivances which are found in warm-blooded animals for maintaining a constant temperature independent of variations in the temperature of the surroundings. The temperature of the plant may be lowered beneath that of the surroundings by radiation and also by transpiration. If we desire to demonstrate the production of heat in the plant it will be necessary for us to retard radiation and transpiration more especially, also to prevent loss of heat by conduction. It will also be necessary to prevent gain of heat by the plant, especially by insolation. Merely by repressing transpiration one may often cause a plant organ, previously exhibiting a temperature below that of the air, to attain a temperature considerably above it. This may be accomplished most easily by selecting for experiment a massive organ with relatively small superficial area, or by heaping together smaller parts and surrounding them with a bad conductor. Thus the temperature of many inflorescences exceeds that of the air very considerably, often as much as from 5° to 10° C. If germinating seeds, growing points, or flower-buds be collected in a flask, and surrounded by a bad conductor, and if special care be taken that oxygen gas can enter in sufficient quantity, considerable rises in temperature

may easily be obtained ; on the other hand, if these structures be dead, and if micro-organisms be excluded, no such rise of temperature is observable even though all the other conditions be maintained. If it be desired to measure minute differences of temperature in individual organs the best method to employ is the thermo-electric pile, viz. copper and iron wires in the form of needles, soldered together, and varnished over, the one stuck into the part to be investigated, the other being in the air or in another organ for comparison ; the apparatus is connected with a galvanometer, from the movements of whose indicator the difference in temperature between the needles may be readily determined (DUTROCHET, 1840).

This method is merely *qualitative* and shows whether heat is developed or not; the *amount* of heat produced can be estimated only by minute calorimetric investigations, the carrying out of which presents many difficulties. According to G. BONNIER (1893) 1 kg. of germinating seeds or young seedlings can evolve twenty, fifty, or even a hundred calories or more per minute. This is a quite considerable amount, for one calorie is the amount of heat necessary to raise 1 g. of water from 0° C. to 1° C. Both calorimetric and thermometric measurements show that not only the conditions of the plant itself but also those of the surroundings play a great part in heat production. We must base our statements on thermometric calculations, for as yet there are very few calorimetric measurements forthcoming.

The production of heat depends to a remarkable degree on the state of *development* of the organ of the plant under investigation ; generally speaking, growing points and young members produce more heat than the same parts when mature. Still DUTROCHET (1840) was able in many plants to establish that an excess of 0·1° to 0·3° C. over the temperature of the air existed in the mature stem if transpiration were prevented. A similar phenomenon is observable in mushrooms, whilst in leaves and fruits evolution of heat is generally more restricted. There are, however, organs which when *mature* exhibit a maximum production of heat and the highest temperatures of all have been observed in fully-developed parts of flowers or inflorescences. Indeed, it is often sufficient merely to *feel* such organs to be convinced that an evolution of heat is taking place. By means of a thermometer it has been shown that the inflorescences of Palmaceae and Cycadaceae and certain parts of the flower of *Victoria regia* not infrequently possess a temperature 10° or more above that of the air, while in the Araceae much higher temperatures have been obtained. Thus KRAUS (1894) found that a thermometer placed in the large spadices of *Arum italicum* gave a maximum temperature of from 49·2° to 51·3° C., or 33·2° to 35·9° higher than that of the air. In nature transpiration brings about a marked cooling effect, since the plant could not for long have maintained such temperatures as were found. Special adaptations may certainly arise quite generally, for we know of Bacteria which are characterized by their high maximum temperature and such forms produce a considerable amount of heat (COHN, 1893).

Amongst external factors *temperature* itself is entitled to receive special attention, since the production of heat by the plant is not entirely independent of external heat. As in the combustion of carbon, &c., production of heat begins to take place only after a sufficiently high preliminary temperature has been attained. At 5° to 6° C. the buds of *Aesculus* show no development of heat, but at about 20° C. an excess of 0·63° may be obtained. Germinating wheat, which at 11° C. gave an excess of 1·1°, at 15° C. showed a rise of 1·4° C. Systematic research on this question is still required, and detailed investigations are especially needed to determine whether a rise in external temperature above a certain point produces once more a diminution of heat production in the plant.

Not infrequently the evolution of heat exhibits a certain regular periodicity. Thus the young inflorescences of *Arum italicum* show at first about the same

excess of heat as any other plant organ. The great evolution of heat takes place on the opening of the spathe towards evening; this increases rapidly in intensity, reaching a maximum just before midnight. Next morning the temperature has again become equal to that of the air and remains so. In *Victoria regia* the rise in temperature commences about nine hours before the opening of the flower, and rapidly increases to a maximum after the flower opens (i. e. towards evening). Then in the night ensues a reduction in temperature, followed by the attainment of a second lesser maximum on the evening of the second day (KNOCH, 1899). Periodicities such as these have been observed in all cases where the production of heat continues for some time. The maxima do not always occur exactly at the same hour from day to day, but they occur during the *day* hours, before or after noon, apparently never at night. The immediate cause of this periodicity is naturally closely connected with periodic variations in the environment, but in what respect *temperature* operates has as yet not been exactly ascertained.

The relation existing between *respiration* and the production of heat is a close one. The experiment with germinating seeds, referred to above, is successful only if a sufficient amount of oxygen is permitted to enter the vessel. It has also been long known that the consumption of oxygen increases *pari passu* with the rise in temperature, and that it is excessive in flowers and inflorescences, which develop large amounts of heat. GARREAU (1857) carried out accurate experiments on *Arum italicum*, and demonstrated an almost perfect correspondence between the absorption of oxygen and the rise in temperature. On the other hand, ERIKSON (1881) showed that on the exclusion of oxygen, and after intra-molecular respiration had begun, the temperature scarcely exceeded that of the air outside. In *Arum*, for example, he was able to demonstrate in intra-molecular respiration, an excess of only 0·3° over the normal respiratory temperature of 16·5°, and in *Raphanus* seedlings 0·2° C. over the normal 5·7° C. Again, in cases of fermentation conducted under anaerobic conditions, a very obvious increase in temperature occurs in the fermenting substance. ERIKSON found that fermenting yeast, under definite experimental conditions, showed a rise of temperature of almost 4°, while the same yeast gave an increase of only 0·2° when milk-sugar was provided instead of grape sugar; moreover, no fermentation could be induced. This result agrees with that previously established, viz. that growth (and we may assert the same of movement also) cannot be carried on in ordinary plants by intra-molecular respiration only, although, by fermentation, it is quite possible in anaerobes. When we finally remember that increase in respiration (p. 202) has also been observed as a result of the action of traumatic factors (RICHARDS, 1896) we may consider the relation between respiration and heat production as sufficiently well established.

These relationships can be most readily explained by assuming that respiration and the related process, fermentation, are the *sources* of the heat evolved. It has been already clearly pointed out that chemical energy must be released when organic substances are oxidized or other chemical decompositions are effected, and this release of energy affords an explanation of the processes under consideration. It needs no proof to establish the view that the energy so released must in whole or in part appear as heat, since in everyday life we employ this method for obtaining it. Still, it may be asked whether respiration is *sufficient* to account for the amount of heat production which has been observed. This question has been answered only by BONNIER (1893), who compared the actual amounts of heat produced with the theoretical amounts arrived at by calculating the oxygen absorbed or the carbon-dioxide given off. Should BONNIER'S results prove correct—and it is advisable that they should be confirmed—it would appear that more heat is actually produced in the germination of many seeds than can be accounted for by respiration. There are other processes in the plant which might aid in the production of heat, such as the solution of solids, the

and friction, e.g. the friction of water against the walls of the vessels. What part these processes severally play in heat production we do not know, but we cannot be far wrong in assuming that they are only of secondary importance, and that respiration is actually the chief factor in the evolution of heat energy by the plant.

If, however, the total amount of energy in the materials oxidized in respiration, i.e. chemical energy, is released in the form of *heat*, we must revise our previous conception of the significance of respiration. Should respiration be the source of the energy required to maintain vitality, chemical energy cannot be entirely transformed into heat, otherwise it would be possible to replace the energy released in respiration by heat energy obtained from external sources, which, as already seen, is not the case. Other forms of energy must obviously be produced by respiration, which cannot originate in any other way. Heat is only a by-product, it might almost be termed a loss of available energy. This holds at least for the majority of plant organs but cannot be true of all; manifestly, it is not in agreement with the great evolution of heat from many flowers. In *Arum italicum*, for example, the spadix is the special organ in which heat is produced. This spadix, before the flowering-period, consists, according to the researches of G. Kraus (1894–5), of about three-fifths water and two-fifths dry substance, 80 per cent. of which latter consists of carbohydrate. The carbohydrate is, in the course of a few hours, completely used up and evidently decomposed into water and carbon dioxide, while the nitrogenous constituents remain intact. The spadix is, however, a mature organ whose function disappears soon after the flowering-period is over, and which exhibits certain special activities during this rapid combustion, which we cannot conceive of as occurring in growing organs. In this case the conclusion cannot be avoided that the carbohydrate is *exclusively* employed for the production of heat. But that all such collections of material are employed for the production of heat, to the exclusion of all other uses, is a proposition not to be entertained. Hence one is compelled to believe that the heat evolved in *Arum italicum* and in such flowers as produce large amounts of heat is not a useless by-product but a special adaptation for attracting insects, as Delpino (1870), and Kraus (1894–5) have suggested. We may at least consider heat production in flowers as a phenomenon *sui generis*, having nothing to do biologically with heat production in other organs, although, looked at from a purely physiological standpoint, the heat is produced in the same way as it is in ordinary respiration in all plants and all organisms.

Although at present we are, generally speaking, unable to present a complete and comprehensive explanation of the phenomenon of heat production in plants, still, the way to reach such an explanation is clearly indicated. For that purpose it is necessary to make comprehensive calorimetric investigations which alone would afford a basis for conclusions as to the *amount* of heat as compared with the total of energy released in the process of respiration.

Imperfect as our knowledge is of the phenomena connected with the evolution of heat, it is extensive as compared with what we know as to the production of *light* by plants. It is true that luminosity is of much rarer occurrence and hence of less general interest, limited as it is to certain groups of Fungi and Bacteria [Molisch, 1904]. (For literature see Verworn, 1901). The phenomenon is closely connected with vital processes, although we have not as yet been able to isolate from the organism any substance which emits light rays, although, certain non-living compounds are known to be luminous. Luminosity cannot be accounted for by a previous storage of light, for it is quite independent of precedent illumination; luminous Bacteria and rhizomorphs emit light rays in continuous darkness. There can be no doubt that luminosity bears the *same relation to respiration* as heat does, for it is manifested only when oxygen is supplied in abundance. We further know that luminosity

is associated with low temperatures, and that luminous organisms lose their power of emitting light when the temperature is raised, eventually losing it permanently. BEIJERINCK (1890) showed that, in the case of Bacteria, luminosity was dependent on the presence of certain food-stuffs. Since these, however, differed in the case of different organisms, it was impossible to draw any general conclusions on the subject. As to the uses of luminosity in the organisms concerned we know less even than as to its causes; it is not therefore worth our while to discuss more fully the detailed observations which have been made on the subject but at once turn our attention to the third form of energy which exhibits itself in plant life, viz. *electricity*.

It has long been known that it is possible with the aid of an accurate galvanometer or a capillary electrometer to demonstrate electrical currents in uninjured plant-organs. If we place non-polarizable electrodes on the leaf of a suitable dicotyledonous plant, so that one electrode rests on the mesophyll and the other on the mid-rib, a positive current will, as a rule, be generated which passes from the mid-rib to the blade of the leaf. The mid-rib is positive to the leaf-surface, as also to the weaker lateral veins. If two points, symmetrically situated so far as the mid-rib is concerned, be connected, no current is demonstrable, nor does it appear when two corresponding points on a stem are connected. Even if no current at all be observable in the uninjured plant, such a current is at once established if the plant be cut or bruised, the electrode nearest to the wound becoming positive to the one farther away. If the intact epidermis be connected with the transverse section of the leaf a current is set up in the direction of the section. If, however, the epidermis be removed and the exposed surface (or a longitudinal section) be connected with the transverse the current flows from the latter to the former.

In 1878 KUNKEL attempted to prove experimentally that all electric currents in the plant were traceable to one cause, viz. the movement of water. It is quite true that disturbances of electric equilibrium may be occasioned by streaming of water, and, according to KUNKEL'S theory the interesting phenomena just described in the uninjured plant are to be explained by the facts that veins and leaf surfaces are unequally wet, and that when wet electrodes are placed on such regions different water currents are set up. According to this theory the electric phenomena observed would have nothing to do with the plant's vitality but might equally well be manifested by a dead leaf.

KUNKEL'S views have not, however, been able to withstand criticism, and more recent investigations, especially those of O. HAACKE (1892) have demonstrated clearly that the evolution of electric currents in plants is by no means such a simple phenomenon as KUNKEL would make out. The movements of water can undoubtedly cause electric disturbances, but they are not the only, or even the chief, agents in the process. It is possible, as HAACKE shows, to demonstrate electric currents in leaves of aquatic plants, equally wet all over, and, on the other hand, the very active transpiration currents are unaccompanied by any electrical manifestations. On the other hand, electric currents are inseparably bound up with vital activities, for dead leaves do not show normal electric currents at all. Moreover, the electric phenomena are intimately connected with respiration, for the currents at once come to an end when oxygen is excluded, while they are especially prominent in actively respiring organs, such as the inflorescence of *Arum* above alluded to. Differences in electric potential are also related to carbon-assimilation. In non-green organs darkness produces no change on the current, whilst in green organs the current ceases at once when these organs are brought into the dark or when carbon-assimilation ceases (compare KLEIN, 1898). Finally, it may be noted that in plants like *Mimosa* and *Dionaea*, which exhibit active movement as a result of stimulus, the movement is accompanied by electric currents which are quite remarkable and regular in their character (MUNK, 1876; BURDON-SANDERSON, 1888).

From the facts cited we must conclude that differences in electric potential appear in all organs of the plant, wherever adjoining parts exhibit chemical or physical differences, and such differences may exist between parts of the same cell or between individual cells or complete tissues.

Detailed information as to the causes of these electric phenomena is not forthcoming, and we will content ourselves with these scanty remarks, since not even guesses as to the significance of such electric currents can be made (compare BIEDERMANN, 1895).

Amongst the varied activities of the plant the production of mechanical energy is, as we have already remarked, by far the most prominent, and the movements which are the expressions of the expenditure of mechanical energy have been much more accurately studied than electric, thermic, or photic phenomena. The rest of the present course of lectures will be devoted to a discussion of these movements. We are already familiar with some of these movements, for when we dealt with the absorption and distribution of materials we incidentally studied the *movements* of these substances in the plant, a subject we dealt with in several lectures in the first section of this work. We have now to study other movements, e. g. the free locomotion of the lower plants, protoplasmic streaming which takes place in a cell and which is analogous to these movements, and, finally, the innumerable varieties of movement seen in fixed organs. In all these movements the plant has to overcome resistance, internal and external and to do work. Without inquiring more intimately at present into the nature of the various movements we may here appropriately summarize the information we possess as to the source of the energy used by the plant in carrying out these movements.

We must first of all make inquiry with regard to the chemical energy which undoubtedly plays an important part in these movements. It is true that the rôle is partly an indirect one, in so far as without the chemical energy set free in respiration it would be impossible to construct the plant or renovate the apparatus which carries on the movement. But we cannot doubt that the energy released during the decomposition of food material co-operates directly, since the endless manifestations of movement are most intimately connected with respiration and stop short at once when intra-molecular respiration begins in ordinary plants. Having established that fact we need only add that respiration is an indispensable condition of protoplasmic movement and that it furnishes the energy necessary for it (PFEFFER, 1892). Again, we have learned elsewhere to recognize the existence of necessary factors which act as stimuli only. It is a fact, however, that the majority of stimuli also bring to the organism a certain amount of energy, but it is characteristic of these stimuli that their energy stands in no relation to that of the effects they produce. The energy of the stimulus may be much less or much greater than that of the movement released, and the latter is certainly not produced from the stimulus but from the stores in the plant itself. Respiration may also be only a releasing stimulus and it certainly is so in many cases, though it is probable that it frequently has a direct energizing significance, or, in other words, that the chemical energy released may be transformed directly into mechanical energy. It is impossible, however, to demonstrate this view. In treating of respiration we are accustomed to compare the energy evolved with that given off in other cases of combustion. In such cases as, e. g. the burning of wood or coal in a steam-engine, we encounter at first a transformation of chemical energy into heat, and it is the heat in the first instance that does the work. In the plant, however, as we have seen, heat as such, evolved in respiration, plays no great part, since it cannot be replaced by heat produced by other means. But even though exact proof were forthcoming that respiration had a purely energizing significance such proof would still fail to satisfy us in the absence of information as to how the chemical energy is transformed directly into mechanical.

Under these circumstances it is but natural that more interest centres round those other forms of energy which may be considered as the *immediate* causes of movement, simply because they are more clearly and more readily comprehended. It is PFEFFER'S (1892) special merit that he has most exhaustively catalogued the forces which, apart from respiration, enable the plant to carry on work. The following are the forces which operate independently of chemical energy :—

1. The transformation of potential into kinetic energy which takes place in the balance in *tensions*, to which may be referred so many of the slinging movements in fruits, stamens, &c., and which manifest themselves in a variety of ways, e.g. by unequal swelling of different wall layers, unequal osmotic pressure of adjacent cells, &c.

2. By the action of *osmotic* energy which not only brings about movement of food-stuffs but leads to vigorous pressures and tensions in the plant. The osmotic energy of a substance is quite independent of its chemical energy, and hence cannot be derived from its heat of combustion. An example quoted by PFEFFER will make this clear. If we assume that the osmotic pressure in a cell is due to glucose dissolved in the cell-sap then we are dealing with a substance which not only possesses a high osmotic energy but also a significant amount of chemical energy. Suppose, however, that the glucose be completely respired into oxalic acid, in that oxidation the cell-sap loses the chemical energy set free, but at the same time its osmotic energy is tripled, for highly oxidized bodies possessing little chemical energy may yet exhibit large amounts of osmotic energy.

3. Quite independently of chemical energy there are various forms of *surface energy*, such as swelling and surface tension, to which will presently be referred many conspicuous movements in plants.

4. 'Form energy,' e.g. *cohesion*, which should also be referred to here.

5. Finally, we may note the energy of *crystallization* and *secretion*, which frequently plays an important part in the growth of the cell-wall.

The mechanical value of these various forms of energy may often be directly measured in a variety of ways, and are, as we have already said, for that reason, more readily comprehended than chemical energy, whose mechanical equivalent is unknown. We must not forget, however, that although mechanical energy plays an extremely important part in the plant, it is a great mistake to refer all the best known cases of movement to the better known forces and to ignore the others.

This bird's eye view of the forms of energy in the plant demonstrates to us how far we are from having attained a comprehensive insight into the problem of the transformation of energy. This is nothing more than what might have been expected, since in the inorganic world also we have yet much to learn on the subject. We are not, however, in a position to deny the validity of the law of the conservation of energy in the organic world.

In the following pages we will attempt to give a detailed exposition of the various movements manifested by the plant.

Bibliography to Lecture XXXI.

BEIJERINCK. 1890. Mededel. Akad. Amsterdam. Natuurk. II, 7.
BIEDERMANN, W. 1895. Elektrophysiologie. Jena.
BONNIER. 1893. Annal. Sc. nat. VII, 18, 1.
BURDON-SANDERSON. 1888. Phil. Trans. 179, 417.
COHN. 1893. Ber. d. bot. Gesell. 11 (66).
DELPINO. 1870. Comp. HILDEBRAND, Bot. Ztg. 28, 590.
DUTROCHET. 1840. Annales Sc. nat. II, 13, 5.
ERIKSON. 1881. Unters. bot. Inst. Tübingen, 1, 105.
GARREAU. 1851. Annales Sc. nat. III, 16, 250.

HAACKE. 1892. Flora, 75, 455.
KLEIN. 1898. Ber. d. bot. Gesell. 16, 335.
KNOCH. 1899. Bibliotheca botanica, Heft 47.
KRAUS. 1894–5. Abhandl. d. naturf. Gesell. Halle, 16, 35 and 257.
KUNKEL. 1878. Arb. bot. Inst. Würzburg, 2, 1.
[MOLISCH. 1904. Leuchtende Pflanzen. Jena.]
MUNK. 1876. Archiv f. Anat. u. Phys. p. 30.
PFEFFER. 1892. Energetik (Abh. kgl. Gesell. Leipzig, 18).
RICHARDS. 1896. Annals of Botany, 10, 531.
VERWORN. 1901. Allgem. Physiologie. 3rd ed. Jena.

LECTURE XXXII

MOVEMENTS RESULTING FROM SWELLING AND CONTRACTION AND FROM COHESION OF IMBIBITION WATER

MANIFESTATIONS of movement in plants are everywhere apparent to an attentive observer, but these movements are not all of equal interest to the physiologist. Our native plants for the most part cast their leaves and even in some cases their branches in autumn, and these deciduous parts may be carried away for long distances by wind and water. Examples of a similar kind are seen in the case of fruits and seeds, but the distribution of these structures differs in this, that it is *useful* to the plant, and that it is facilitated by special contrivances, e.g. by special wings for distribution by air currents, floats for distribution by water and by hooks for transport by animals. Such movements are, however, effected without any expenditure of energy on the part of the plant, they are purely passive movements, very important, it is true, from the biological point of view, but outside the domain of strict physiology. There are, however, passive movements which do interest the physiologist, such as the downward bending of branches by their own weight or, conversely, the straightening of the branches of submerged plants by water support. In the cell also we have to take into account the passive movements of chlorophyll bodies, e.g. in *Vallisneria*, consequent upon the rotation of the protoplasm, or, in other cases, where protoplasmic movement distributes the chloroplasts in definite situations. Although we have to study in the following pages primarily the active movements of plant organs, still we must not neglect the purely passive ones, all the more so since no sharp line of demarcation can be drawn between the two types. Observation of the gradual bending upwards of a shoot laid horizontally teaches us that it is due to curvature taking place at a certain distance from the apex. This movement may certainly be termed active, since the plant in this case actually does the work, but this work is performed only in a definite spot, at the point of curvature ; the distal end of the shoot is lifted in a purely passive manner.

Under active movements we may distinguish two main categories:—(1) locomotory movements of entire organisms, met with only among the lower plants ; (2) movements exhibited by higher plants which grow in fixed positions. This distinction is not a hard and fast one, since the protoplasm within the cells of a higher plant moves in precisely the same way as the entire organism does, say in the case of Amoebae or Myxomycetes; indeed, in certain cells it may pass out of the cell-wall and move through the water for a certain time, just as do the cells of Flagellata during their entire life. Many analogies between these two categories of movements are forced on our attention, and so, obviously, the classification we have indicated is to be considered not as expressing a fundamental difference in nature, but rather a useful subdivision for teaching purposes.

Movements in fixed plants came under our notice in another relation, as for example, when the root or the stem apex grows forwards in process of development, and although such orthotropic movements are not those which, as a rule, we have in mind when we speak of movements in plants, still it is impossible to separate such movements as these from movements in general. The first type of movement we naturally think of is the bending of an originally straight organ or the alteration in curvature of an organ showing curvature to begin with. We have now to study such curvatures both as regards their form and their causes, and we may begin by considering such movements as appear in ripening fruits or other drying parts, and whose cause lies in the loss of water from the cell-membrane—in other words, hygroscopic movements.

The changes of shape which an organ thereby undergoes may be referred to one or other of three fundamental types: thus we may speak of mere *curvature*, when an originally straight organ becomes bent so that its axis lies in one plane; of *twisting* when the axis of an organ retains its original direction, while the longitudinal lines of growth from being straight become spirally twisted; and, finally, of *twining*, when the entire organ becomes altered into the form of a spiral. Fig. 119 shows these three conditions in the case of a quadrangular prism, and might also represent to some extent similar changes of form in the case of a stem.

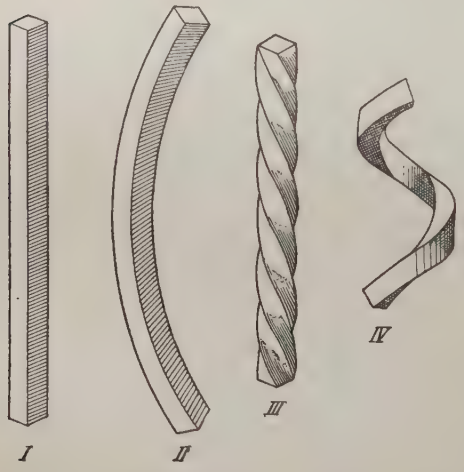


Fig. 119. Quadrangular prism. *I*, straight. *II*, bent. *III*, twisted. *IV*, twined.

That the cause of the deformation in such organs lies in the loss of water during desiccation is shown by the fact that they regain their original shape when moistened, and that the one or the other condition may be induced at will according as water is added or withdrawn. The capacity for absorption of water is widely distributed in the vegetable kingdom and may be due either to the osmotic activities of the cell-sap, or to the power of imbibition possessed by the different parts of the cell. Hygroscopic movements arise from the latter capacity, and especially from the imbibition of water by the cell-wall; indeed such movements take place even though the parts under consideration consist of cell-walls only. We have several times referred to this phenomenon of imbibition, but now is the proper time to study it in somewhat greater detail.

We must first ask ourselves wherein lies the essential essence of the imbibition. Bodies which are capable of swelling are able to absorb a liquid, and thereby to increase in volume; but it is obvious that this imbibition of liquid must, to a certain extent, be limited, and that it brings about for the most part an alteration in the consistence of the swollen body. In plants, water is the medium which especially induces swelling, a medium which can also produce swelling in other bodies which do not occur in plants. If we take a piece of gelatine or glue, and determine its weight, and then lay it in water at an ordinary summer temperature, we can observe an absorption of water as well as an increase in volume, and with the aid of a balance we may convince ourselves that the absorption takes place with decreasing rapidity and finally ceases. If the

water be warmed, more will be absorbed, and we discover that the power of absorption possessed by gelatine depends directly on temperature. When the temperature reaches a certain degree the absorption becomes unlimited, or, as we are accustomed to put it, the gelatine dissolves in the water. The same thing applies at ordinary temperatures to gum arabic—the absorption of water by that substance suggesting at first a case of swelling, but becoming indefinite. Hence we see that swelling may gradually merge into solution. It would be quite incorrect, however, to assume that all bodies capable of swelling are also capable at some definite temperature of dissolving in the medium employed. Cell-walls especially, at the present moment of primary interest to us, remain stationary when they reach a state of maximum imbibition. The changes therefore which gelatine undergoes in solution we need not discuss.

In order to obtain a closer insight into the phenomenon of water imbibition we will compare substances capable of swelling, such as a cell-wall, or a piece of gelatine with a finely porous body, such as a plate of plaster of Paris saturated with water and afterwards air dried. If a plate of plaster of Paris be placed in water, it absorbs a certain quantity of it and retains it firmly when withdrawn from the water. The water is, however, retained in pre-existing spaces, as may be seen from the fact that the air escapes in bubbles when the plaster is placed under water ; in other words, the water forces its way into the plaster by capillarity and replaces the air previously present in these spaces. In a cell-wall or a piece of gelatine, on the other hand, capillary spaces containing air cannot be seen even with the best lenses, and, further, impermeability of the substance to air proves that such spaces do not exist. Even were such spaces present there is yet another fundamental difference between finely porous bodies and bodies capable of swelling. The plaster of Paris shows no increase in volume after the absorption of water, such as substances do which are capable of swelling. Further, when water forces its way into previously existing spaces in the latter, these spaces must be enlarged by the water and the minute particles separated from each other, a phenomenon which obviously does not take place in a solid body which does not increase in volume. On the contrary, ASKENASY (1900) has observed that in consequence of a capillary entrance of water a *diminution* in the volume of a non-swelling body may take place, e. g. in deposits on cover-glasses. Bodies capable of swelling must possess a special molecular structure which may not be directly observable but which can only be deduced from observation of their behaviour.

Among hypotheses of molecular structure, that advanced by NÄGELI has undoubtedly had the most lasting influence in Botany, more especially in relation to the phenomena of imbibition, and even now it claims a certain recognition. Since, however, some essential parts of the hypothesis have been refuted, it will serve our purpose best if we deal here merely with such parts as are vital to our discussion. NÄGELI (1858) held that bodies capable of swelling were composed of extremely minute particles, larger than molecules, to which he gave the name of micellae. Since there is no longer any ground for believing in the existence of micellae we need not lay any emphasis on this conception. When the body was in the dry condition the micellae were supposed to be close to each other without any air-spaces between them ; the micellae were regarded as polyhedral in form, and were considered to be held together by mutual attraction. Since the micellae had also an attraction for water, each attempted to surround itself with a film of water. This, however, could not take place unless the force of attraction between water and micella, was greater than that between the micellae themselves. The addition of water thus induced a separation of the micellae, and explained at once the increased volume of the swollen body. Should this be limited, the resistance offered by the separation of the micellae to any further entry of

water must rapidly increase. NÄGELI suggested that the force of attraction between the substance and the water decreased relatively more rapidly than that between the micellae, the former being more difficult to separate in inverse proportion to the latter. Thus all the water in the swollen body was not held equally firmly. The particles nearest to the surface of the micella were held most firmly, and as the distance from it increased the mobility of the water also increased, and it was very probable that not all the water imbibed lay within the spheres of attraction of the micellae, but was retained as capillary water in minute spaces arising during the swelling. In fact, REINKE's (1879) researches on *Laminaria* have shown very clearly that imbibition water is retained with varying tenacity. In these experiments certainly larger spaces, filled with capillary water, e.g. the cell lumina, play a part. REINKE allowed a portion of the blade of a *Laminaria*, which had absorbed 1·026 g. of water, to dry in air, and found that it evaporated the following amounts in mg. in successive hours : 148, 115, 105, 91, 74, 84, 68, 57, 51, 51, and, later on, still less. Further, it is possible to express water from a completely swollen portion of *Laminaria*, containing a large quantity of water, with only slight pressure, but great pressure is necessary to extract water when it is present in small quantity. If a *Laminaria* consists of 75 per cent. water and 25 per cent. solid, water may be pressed out by a pressure of two atmospheres ; if the proportion of water to solid be 43 per cent. to 57 per cent., a pressure of forty atmospheres is needed to achieve the same result. Again, the swelling may be prevented by great pressure, so that it is very obvious that a great deal of work is accomplished in the process of swelling. RODEWALD (1895) showed that a pressure of twenty-five to thirty-two atmospheres is needed to prevent dry starch from swelling, and it is known that mechanical operations may be carried out by the swelling of certain bodies, e.g. rocks may be split open by the swelling of wooden wedges, and a skull may be separated into its constituent bones by filling it with peas and allowing them to absorb water. Under these circumstances we may assume that air-dry substances capable of swelling always retain demonstrable quantities of water, and that they are able further to condense water vapour from damp air.

Simultaneously with the absorption of water a noticeable alteration takes place in the mechanical characters of the swollen body. If the substance when dry be brittle and only slightly extensible, when swollen it may become pliable and very markedly extensible, and yet it loses its elasticity and its rigidity (under tension and pressure). It is important to note how great are the quantities of water which may be absorbed by a body without entirely losing its rigidity, and without transforming it into a liquid. According to NÄGELI the gelatinous cell-walls of certain lower Algae contain only one-half per cent. of dry substance ; but even that is far from being the extreme limit that may be reached, since, according to GERICHTEN (1876), apiin, a glycoside obtained from parsley, begins to lose the capacity for forming jelly only when one part of solid is dissolved in more than 8,000 of water. It is not easy to understand how the characteristic features of a solid are preserved when the individual molecules are separated so far from each other as they are in the cell-walls of the Algae mentioned above, where the molecules must be separated by a distance equal to many times the diameter of the molecule. These considerations force us to accept some other theory of structure than that hitherto held. What we need is a structure which maintains its cohesion sufficiently well even when large quantities of water are imbibed. We should have such a structure if we assume the substance capable of swelling to be permeated by canals so that it consisted of minute particles bound together in all directions, just like the meshes of a net in a plane, or, better still, regard it as the honeycomb of soapsuds, where the walls are formed of a substance capable of swelling while the alveoli are able to take in water. Honeycomb constructions of this kind have been shown by BÜTSCHLI (1892–

1900) to be of general occurrence in all bodies capable of swelling both in the cell-membrane and in the protoplasm. He lays stress on the fact that the diameter of the spaces is uniform, amounting to about one μ. Certainly, in protoplasm these very minute but visible cavities show all transitions to the large vacuoles. BÜTSCHLI holds that the cavities contain a dilute solution of the swellable body which is concentrated by loss of water and thus acts *osmotically*. The expansion of the walls of the alveoli would thus be due to osmotic pressure. In addition to other difficulties this conception is open to the criticism that cellulose is quite insoluble in water. BÜTSCHLI does not, however, hold that during the process of swelling the stretching of the walls of the cavities is due *only* to the pressure of their contents ; he expressly shows that water is also absorbed by the *walls of the cavities* themselves, a point in which substances capable of swelling differ from those incapable of doing so ; at the same time it must be remembered that the latter also may possess a honeycomb structure. The absorption of water by the walls of the cavities is considered by BUTSCHLI to be a *chemical* phenomenon, a case of hydration in fact, and he thinks that this water cannot be got rid of merely by pressure, and, further, that the water expressed in REINKE's experiments above described was only water from the cavities of the honeycomb and from the larger spaces in the substance. We may, however, quite well make use of NÄGELI's *physical* hypothesis for the imbibition of water by the walls of these cavities and so combine his theory with that of BÜTSCHLI. Hence it is worth noting that (see p. 407) the existence of intermicellar spaces, corresponding to BÜTSCHLI's alveoli, had already been considered by NÄGELI (1858, 342).

No matter which theory be the correct one, the walls of the cavities must be increased by the imbibition, and the cavities must thus be able to hold more water, itself out of reach of the attractive force of the micellae. BÜTSCHLI's observation that in the process of drying the walls of the cavities collapse and approach each other until the lumina entirely disappear is of the utmost importance. The full significance of the disappearance of the alveolar structure in drying and its reappearance on water being once more absorbed will become evident later on when we have studied the phenomena of cohesion (p. 417).

The alterations in volume associated with swelling and shrivelling permits of the execution of movements on the part of such bodies, and this leads us back to the hygroscopic movements we started with. If the object under consideration is capable of swelling equally in all directions, then it or its parts will be able to exhibit movements only in straight lines, but such movements are of no further interest. The bending, twining, and twisting of hygroscopic organs can obviously be produced only if the capacity for swelling varies in different directions, when layers with greater powers of imbibition stand in antagonism to those with less capacity for swelling. We distinguish the layer which contracts most as the ' contractile ' or ' dynamical ' layer, and that which does so least as the 'resistant' layer. Variations in the capacity for swelling are due, in the first instance, to the varied nature of the material, in the present instance the cell-wall, generally put down to chemical differences but assumed by NÄGELI to be physical, and especially dependent on the varying size of the micellae. On the other hand, the structure of the membrane may render possible differences in capacity for swelling in different directions. NÄGELI's micellar theory, as also BÜTSCHLI's alveolar theory, equally well explain such unequal swelling. We will consider the observations themselves without going into theories with regard to them, and we find, speaking quite generally, that a cell which is not isodiametric is unequally extensible in its three chief space dimensions. The greatest capacity for swelling in an elongated cell is in a radial direction, i.e. at right angles to the concentric layers of which its wall is composed ; it has less capacity for swelling tangentially, and least of all longi-

tudinally. Its behaviour during contraction naturally corresponds. If we think of a point in the interior of the swollen cell-wall as on the surface of a sphere, this surface changes during drying to an ellipsoid form ('contraction-ellipsoid') whose shortest axis is at right angles to the lamellation, while both the other axes come to lie in a tangential direction. But it is not necessary, as has been pointed out, that the longest axis should be coincident with the greatest length of the cell, it may lie obliquely or transversely. In most cases it would be very difficult to determine directly by measurement the lie of the axis of contraction during desiccation, and hence it is of importance to be acquainted with indirect methods of doing so. In the first place, we may draw attention to polariscopic research, which lends itself to the determination of optical elasticity-ellipsoids. Experience teaches us that this almost always corresponds with the lie of the axis of the contraction-ellipsoid. In the second place, the direction of the thickening bands, of the striations, and pits must be noted, since that corresponds to the position of the longest axis of the ellipsoid, and conforms to the line of least contraction in desiccation. The position of the longest axis may, however, be different in the different walls of the same cell; for example, it may run longitudinally on the outer wall and transversely on the inner, and may alter in successive layers.

It will not be necessary for us to discuss hygroscopic movements either generally or in detail, for that would involve us in difficulties, due to the fact that all authors do not agree as to the interpretation of the more complex cases. Only a few examples need be cited here as illustrative of the chief types (for the older literature see KRAUS, 1866; HILDEBRAND, 1873). We begin with the consideration of simple bendings such as we meet with in *Anastatica hierochuntica*, the Rose of Jericho, a member of the Cruciferae from the Steppes of the south-eastern Mediterranean area. When the fruit is ripe the numerous divergent branches dry up, and in doing so contract much more on the upper than on the under sides, thus bending inwards and causing the plant to take on a spherical form. When moistened they again open out, and this performance may be repeated again and again. Movements of the fruits take place at the same time, but into these we need not go. It is easy to show that the bending is due to the wood only, and anatomical investigation of a twig shows (VOLKENS, 1884) that this consists especially of excentric xylem fibres, much more thickened and lignified on the under (convex) than the upper sides. The strongly lignified fibres are much less capable of absorbing water than are the feebly lignified ones, and hence on desiccation the upper side of the branch contracts much more markedly than the under side. The bending in this case is due to the differential capacities for swelling of antagonistic tissues. In the same way (STEINBRINCK, 1878), each of the five mericarps in the fruit of the geranium bends outwards after drying, releasing itself with a jerk from the central carpophore, and so aiding in the dispersal of the seeds.

The bursting of many forms of capsule is brought about in a similar manner. Some part of the fruit-wall endeavours to bend outwards and the tensions set up finally cause a rupture in the region of least resistance, frequently at places where there are special anatomical structures, differentiating lines of dehiscence. The cause of the tension, for the most part, does not lie in the different intensity of imbibition of antagonistic zones but in the layering of the cells, or, in other words, in the direction of the lamination or striation of the wall. The various possible arrangements may be illustrated by a few examples.

1. *Differential contraction due to arrangement of cells.* In the walls of the segments of the capsule of *Syringa* we find a lignified layer which is the sole cause of the bending, and which consists of six rows of elongated thick-walled cells; the innermost of these are arranged longitudinally, while the outer layers are deposited obliquely and transversely. Since these cells are all alike, in so far as their capacity for absorbing water is concerned, bending when

desiccation sets in, must be due to the differences in their mode of deposition. The cells contract relatively less in a longitudinal direction than transversely, hence the outer sides of the segments contract more vigorously than the inner sides, and the segments become concave outwardly. It is not, however, essential that the elements of both the antagonistic layers should be, as in *Syringa*, elongated and cross each other at 90° or less ; it is sufficient for the purpose if one cell layer be composed of fibres while the other is formed of isodiametric cells. Thus in the wall of the fruit of *Veronica* we find the epidermal cells of the interior are thick-walled fibres, whilst externally there lies a layer of parenchyma capable of contracting equally in all directions (STEINBRINCK, 1878). Differential contraction between this layer and the inner epidermis is greatest in the long axes of the latter elements, and must, therefore, cause a bending outwards at right angles to the course of these elements.

2. *Differential contraction due to lamination of the cell-wall.* As an example of this type we may select the capsular teeth of *Linaria* (STEINBRINCK, 1891). Fig. 120, *1*, shows a part of a median longitudinal section of that portion of the tooth specially concerned in the contractile movement, that is, through the inner epidermis and the sclerotic layer abutting on it. The cells figured are, it is true, by no means isodiametric, nevertheless the important factor in the bending is not the arrangement of the cells but the lamination of the cell-membranes. The two cell-layers differ essentially in the way in which their cell-membranes are deposited. The lamination of the inner epidermis is almost perfectly parallel to the long axes of the capsular teeth, and the same is true of the inner walls of the sclerotic layer. In the remaining portion of the latter one would expect a similar arrangement, in accordance with the usual principles of unilateral thickening, that is to say, a deposition of secondary thickening in layers tangential to the outer surface as in Fig. 120, *2*. As a matter of fact, however, a glance at Fig. 120, *1*, shows that all the lamellae are laid down parallel to the horizontal walls. Since the maximum contraction, as we have already pointed out, occurs at right angles to the lamination, the greater part of the sclerotic layer contracts much more markedly in the long axis of the capsule than the inner epidermis and the inner wall of the sclerotic layer. Measurements made on the isolated layer exhibited a shortening in the former of 10 per cent., while in the latter the contraction was scarcely observable.

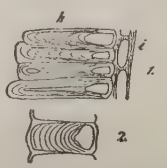


Fig. 120. *1*, longitudinal section through a valve of the capsule of *Linaria vulgaris*. *i*, inner epidermis; *h*, sclerotic layer. × about 300. *2*, external epidermal cell of *Dianthus prolifer* in transverse section. × about 150. After STEINBRINCK (1891).

3. *Differential contraction due to striation in the cell-wall.* We may briefly characterize the last case we took by saying that in it the difference between the shortest and a longer axis of the contraction-ellipsoid is made the most of. In contrast to this we find cases in which the difference between the longest and the average axis comes into play, the cases in which the bending is due to striation of the membrane. (*a*) The capsules of *Campanula* (STEINBRINCK, 1895) open in the same way as those of *Linaria*, viz. by valves, but the histological structure and the mechanism of opening is quite different in the two plants. The sclerenchyma is absent from *Campanula* altogether, and the bending is brought about by the parenchyma, and is due in part to the form of the cells, the external layers being composed of short cells gradually increasing in length inwards. Any bending must therefore take place so that the concavity is external, in accordance with the principles already laid down. A second factor is the striation of the cell-wall which expresses itself in the position of the pits. The outer cells have their pits arranged transversely, while on the walls of the cells of the succeeding layers the pits are laid down obliquely to the left and finally longitudinally. Since, as we have seen, the long axis of the contraction-ellipsoid

lies parallel with the long axes of the pits, the average axis of contraction is made the most of in the dynamical external cells, and it operates in opposition to the longest axis in the innermost cells, forming the resistant layer. (*b*) A difference in the striation of the wall of a single cell may also occur, as in *Saponaria*, where in the dehiscence of the capsule we have merely to deal with the external epidermis, whose greatly thickened outer walls act as the contractile layer, whilst the radial and inner walls serve the purpose of the passive layer. But, contrary to one's expectation, the pits on the outer walls are not transverse, nor are those on the inner wall longitudinal; the difference is of quite another character. According to STEINBRINCK (1891) the inner wall bears numerous clearly marked, narrow, elliptical pores, transversely placed, while on the outer wall the pits gradually become less distinct, less numerous and more elongated and, finally, in this region of maximum curvature, fade away into dark narrow streaks running transversely from one radial wall to the other, alternating with clear striae. It is easily seen how, by this arrangement, the differential contraction is effected, for the inner wall with its short transverse pores contracts far less than the outer wall. It should be noted that STEINBRINCK (1891) found an extreme case in *Dianthus prolifer*; here the most external layer of the outer walls of the epidermal cells acts dynamically, while the innermost layers of the same cell-walls are the resistant ones, that is to say, the antagonistic units are parts of the same cell-wall.

Fig. 121. Pod of *Orobus vernus*. After KERNER (Pflanzenleben, 2, p. 773).

The way in which we have treated this subject might lead to the supposition that in each individual case of hygroscopic bending only one or other of these three principles came into operation, i. e. qualitative differences between the imbibitory capacities of the cell-wall, differences between different layers, or differences in striation. That is, however, not the case; as a rule, combinations of these possibilities occur in nature, and it is only for brevity's sake that we have avoided treating of such in individual cases.

Let us now turn from *curvatures in one plane* to the more complex phenomena of twinings and torsions (compare NÄGELI and SCHWENDENER, 1877). In these cases also the same structural principles are applicable, but we must resist the temptation to discuss these in as great detail as we have the simple bending movements. We meet with very noticeable twistings in the two segments into which the pods of Leguminosae divide on ripening. The general nature of this twisting is illustrated in Fig. 121, where the inner surface of the fruit wall maintains its internal position during twisting. Anatomical investigation shows that the inner epidermis abuts on a sclerotic layer, which alone is the factor concerned in the twisting (ZIMMERMANN, 1881, p. 25). All the cells of this layer are elongated, but the innermost have great powers of contraction (15 per cent.) while the outermost have none at all. It is possible to determine anatomical differences between these fibres, but probably it is not to these differences but to certain chemical differences which have not as yet been elucidated that the varied behaviour of the cells is to be attributed. If these fibres lay parallel to the long axis of the pod both segments would simply bend inwards in a concave manner, but, as a matter of fact, they lie at a sharp angle with the long axis of the legume, and thus the curvature which is transverse to the direction of the fibres is also oblique to the long axis of the pod. Let us assume a long narrow piece of paper folded obliquely, as represented by the dotted lines in Fig. 122, it will take the form of a spiral when pulled out. Although, as ZIMMERMANN has shown, the sclerotic layer is alone sufficient to

Fig. 122.

bend the pod, the external epidermis is able to aid in the process, as STEINBRINCK (1873, p. 17) has suggested. The epidermal cells are elongated and traverse the fibres, so that it is obvious that a differential contraction must occur between them. In relation to what follows it is necessary to emphasize the fact that twistings must result from the reactions between epidermis and fibres, since differences such as those found by ZIMMERMANN do not occur among the fibres themselves, all the fibres behaving exactly alike.

Of even greater interest is the spiral coiling exhibited by the lower parts of the awns of *Erodium* (Fig. 123, *A*), for there the twisting takes place obliquely to the long axis of the fibres of which the awn is composed—for the epidermis and parenchyma need not be taken into account, having no power of hygroscopic movement. The thick-walled fibres are differentiated into four layers which gradually merge into each other from without inwards (STEINBRINCK, 1895) :—

1. A layer of fibres with transversely placed pits, which when isolated bend outwards only slightly on drying.

2. Fibres provided with pits which lie transversely or are slightly tilted upwards to the right on the outer walls and with pits on the inner walls tilted upwards considerably to the left. The whole layer and each individual fibre when separated from the other layers twists on drying just as the whole awn does.

3. Fibres with pores arranged longitudinally; these fibres on drying do not bend at all.

4. A layer of fibres which when isolated and dried twists to the right, i. e. in the opposite direction to that of the awn as a whole.

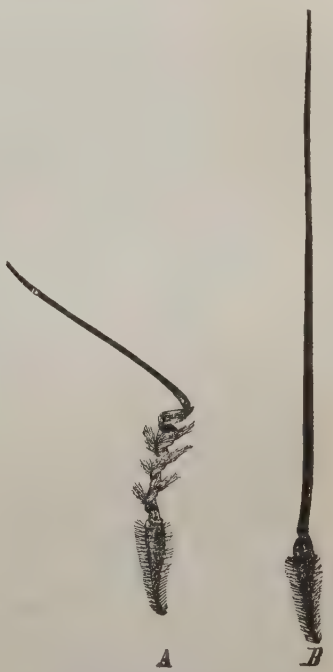


Fig. 123. Portion of the fruit of *Erodium*. *A*, in the dry state; *B*, in the wet condition. From the Bonn Textbook.

It will be obvious from what has been said that to the second layer only or to that in opposition to the other layers may be attributed the twisting of the awn. Every individual cell in it endeavours to twist on its own account, and it is not difficult to see how the twisting as a whole may be explained if we compare the individual fibres of this layer with the pods of the Papilionaceae. The transverse or feebly oblique pores directed to the right on the outer walls of these cells indicate to us which is the long axis of the contraction-ellipsoid; they correspond in their deposition to the epidermal cells of the legume. As regards the inner wall, the axis of the ellipsoid, as in the legume, lies almost transversely to that of the outer walls. It is easily intelligible not only how the second layer as a whole twists, owing to the efforts to twist of the individual cells, but also how the first and third layers merely intensify this twisting. As a matter of fact, it has been observed that the second layer twists whether in conjunction with the first or the third or with both, and, finally, the fourth layer must be added whose endeavours to twist in the reverse direction are completely neutralized by the others.

The last-mentioned layer behaves differently from the others for other reasons. In layers 1–3 we have to deal with an antagonism between flat intersecting plates, i. e. between layers whose axes of contraction intersect each other (STEINBRINCK, 1888). These plates are variously distributed :—(*a*) they occupy

the outer and inner walls of *one* cell (layer 2); (*b*) they occur in different cells; or (*c*) both conditions are united. Under all conditions the radial cell-walls have transverse pores and play no part in the twisting. In the fourth layer the pores are laid down in continuous spiral lines directed to the right, running from the front wall, over the lateral walls, to the rear walls; in other words, we have here to deal not with two intersecting plates but with elements which are spirally arranged. If the wall of such an element be equally capable of swelling, each must, as ZIMMERMANN has shown, twist independently. How a complex of such cells bound into a tissue may bring about a spiral twisting may be easily shown by experiment. Cover a strip of paper thickly with portions of the twisting awn of *Stipa*, gum these to the paper and to each other, and leave the whole to dry; the structure will then exhibit a close left-hand twining with the paper external (STEINBRINCK, 1888, p. 392).

This last example illustrates the close relationship which exists between twisting movements and the *torsions* which we have now to discuss. We have seen that the individual cells must undergo torsion if they are provided with pits arranged in a spiral manner. An *entire organ* can also undergo torsions similar in all respects to those exhibited by its constituent elements. DARWIN (1876) bound together wet awns of *Stipa*, and found that the bundle underwent torsion when it was allowed to dry, but apparently this principle does not come into play in nature (*Anemone*? EICHHOLZ, 1885, 554). Torsions of the whole organ occur much more frequently in consequence of the tendency to twist on the part of the individual elements when these are arranged in concentric layers. Such torsions must arise from a relatively greater extension in the peripheral than in the central region. If we twist a bundle of fibres laid parallel to each other we shall find that each, with the exception of the central ones, describes a spirally curved line, and conversely if twisting of the individual elements occurs there arises torsion in the bundle as a whole. The twisting cells of *Stipa*, which have the structure of those composing the fourth layer in *Erodium*, are the chief agents in the movement, and one important condition only must be fulfilled, viz. that the fibres should exhibit an increasing capacity for water absorption longitudinally from without inwards, so that in drying the central ones should contract more than those on the periphery.

A few words may be said in conclusion on the biological significance of the movements which have been described. Almost all of them have to do with the dispersal of seed. In the great majority of cases fruits burst open or dehisce when dried, part of the fruit wall being thrown off, and the seeds escaping in this way from the capsule. There are many plants, however, such as *Anastatica*, *Mesembryanthemum*, &c., whose fruits close in dry weather and open in wet. Fruits which have the power of ejecting their seeds form more perfect illustrations of these hygroscopic movements; examples of such are *Geranium*, the twisting pods of Leguminosae and many others which have not been referred to above, such as *Viola*, *Oxalis*, &c. Fruits and seeds which have long twisting awns, such as *Erodium*, *Stipa* (and many other grasses), many species of *Anemone*, &c., are able to force their way into the soil by the torsions which take place in these appendages.

Let us now turn to the consideration of a series of phenomena which are illustrated by the movements of anthers and sporangia, connected with the ejection of the pollen-grains or spores, and hitherto briefly spoken of as hygroscopic movements. We shall consider first the sporangia of ferns, and especially those of the Polypodiaceae. The sporangium is a stalked, lens-shaped body, enclosing the spores within a multicellular unilamellar wall. Most of the cells of the wall are polyhedral, thin-walled plates, but the edge of the lens is occupied by a ring (annulus) of horseshoe-shaped thick-walled cells, which starts from the stalk and more or less completely encircles the capsule (Fig. 124, *a*).

The inner tangential walls of the cells of the annulus are strongly thickened, but the outer walls are unthickened, while on the radial walls the thickening is gradually reduced from within outwards. When the sporangium is ripe, at the region where the annulus ceases, or, to be more exact, where the cells of the annulus cease to have thickened walls (Fig. 124, *1*, *st*), there occurs a rupture due to the contraction of the annulus, so that the sporangium takes the form seen in Fig. 124, *2*, and the spores can thus escape. Obviously in consequence of the loss of water from its constituent cells, the annulus contracts and bends slowly outwards, and this contraction and bending may proceed so far that the annulus again forms a circle, but now what was the inner face becomes the outer. At this moment a new phenomenon appears, for with a sudden snap the annulus springs back and once more assumes almost its original position and form. It rebounds on its base with considerable force, and the whole sporangium is thrown often several centimetres into the air, and in the process the spores which still adhere to the capsule are ejected. Looking more closely at the annulus during the opening of the sporangium we see that its cells undergo a remarkable deformation. The water they contain evaporates, and the cell cavities become smaller, the thin external walls become concave inwards while the lateral walls approximate. The external outline of the annulus thus becomes gradually shorter, and the inward curving of the ring is thus simply explained. The *curving inwards* of the outer walls of the individual cells of the annulus and the *water they contain* proves clearly that this is not a case of 'shrivelling'.

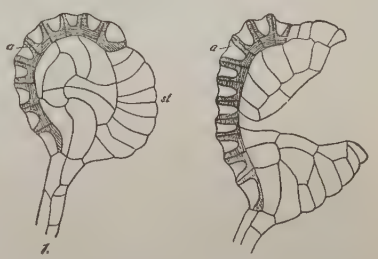


Fig. 124. Closed (*1*) and open (*2*) sporangia of the Polypodiaceae. *st*, lip-cells; *a*, annulus.

Inquiry as to the factors which bring about the ultimate deformation of the cell whereby the outer wall approximates to the base of the cell and the outer corners of the radial walls come to touch each other, shows us that we must investigate the cohesion of the imbibition water of the cell and its adhesion to the membrane (STEINBRINCK, 1898, 1903). In speaking of the movements of water in the plant we have already shown that the cohesive force of water is very great. The adhesive force of water to the membrane is almost as great—inasmuch as the tension of several atmospheres is required to tear the water particles apart from each other or from the wall. When evaporation commences, therefore, the water in the cells must be under tension and the effect of that tension is to produce deformation of the cell. If the cell-wall could not be deformed the water cohesion would soon be overcome and an air space would appear in the interior of the cell, or a rupture would occur between the water and the membrane, and air would at once enter. It was previously thought that such movements as those seen in the fern annulus (and which we may term cohesion movements, in contradistinction to those due to swelling and contraction) could only take place if the cell membrane were impermeable to air. If that were the case their occurrence would be very restricted, and they could not in any case take place in the fern sporangia since the outer walls of the annulus cells are, as a matter of fact, quite permeable to air. The entry of air into the interior of the cell is, however, rendered impossible at first, inasmuch as each minute bubble of air must first of all overcome the adhesion of the water to the cell-wall.

The imbibition water in the annulus cells exercises in this way during evaporation a vigorous pull on the walls, and stretches them elastically. Finally,

this tension becomes so great that it overcomes the cohesive force of the water particles, the water continuity in the interior of the cell is ruptured, and the cell membranes assume once more their original form, and the annulus bends back again with a sudden jerk into its original position of rest. Its cells now appear dark, for they contain only a little water distributed on their walls, and for the rest a space which may in general terms be said to be full of air. The entry of air is not, however, essential to the execution of the elastic recoil, since this recoil occurs when the sporangia are placed under low pressure in an air pump (SCHRODT, 1897) ; in that case a *vacuum* appears in the centre of each cell of the annulus after the recoil.

The mode of opening of the anthers of Phanerogams corresponds in all essential points, according to STEINBRINCK's researches (1898, 1899 a), with the mode of dehiscence of fern sporangia. Each of the four lobes of the anther, filled with pollen-grains, consists of a wall which in the ripe condition is often only two cells thick. The pollen-grains are released in consequence of the power the wall has of curling backwards. In this process the outer cell layer of the anther wall takes no part ; the inner, usually known as the fibrous layer, holds the dynamical elements. The thread-like thickenings on the inner walls of these cells are laid down in a very characteristic manner (Fig. 125). They run almost parallel with each other at regular distances apart, over more or less of the surface of the lateral walls, uniting on the inner wall like the rays of a star ; the outer wall is destitute of any thickening. The comparison with the fern annulus is perfectly obvious, but the fact that the lateral walls are in this case unequally thickened results in a difference in behaviour between the anther and the fern sporangium. The fibrous cells also undergo deformation during the loss of their imbibition water on drying. The deformation consists, in the first instance, in the shortening of the diameter of the cell on the external face of the anther, while that of the inner side remains rigid owing to its secondary thickening. The change in form does not in this case express itself in an inversion of the outer wall, but in a very noticeable contraction of the radial walls at right angles to the lines of thickening, so that these bars approximate (Fig. 125, *IV*). This contraction, according to SCHWENDENER (1899) and STEINBRINCK (1901) may amount to 50, 60, or 70 per cent. of the original diameter. Were this the result of simple shrivelling the contraction would far and away exceed that shown by any other cell. But STEINBRINCK has shown that the contraction begins while the cavity of the cell is still full of water, and hence it is obvious that it cannot be due to mere shrivelling.

Fig. 125. Anthers of *Lilium candidum*. *I*, isolated fibrous cell in the wet state. *II*, the same as seen from without. *III*, as seen from within. *IV*, as seen in section after drying. After STEINBRINCK (1895). × 150.

As a matter of fact, the process must be explained in an entirely different manner. Under the influence of the tension exerted by the imbibition water the thin parts of the radial wall lying between the thickening bars are thrown into folds, and hence the volume of the cell is reduced. Apart from these foldings, which may be best observed in good tangential sections through the anther, there is another difference to be noted between these cells and those of the fern annulus. At the moment when the elasticity of the bent fibres overcomes the cohesion of the imbibition water, when a bubble of air appears in the interior of the cell, no jerk takes place as in the annulus, but the anther wall remains in the outwardly bent concave condition. The reason for this is probably that the thin-folded portions of the wall adhere to each other, and only become

smoothed out again when water is reabsorbed ; then only does a closing movement of the lips of the anther take place.

It must be admitted that the mechanism of the anther has not as yet been so completely explained on the basis of the cohesion hypothesis as that of the fern sporangium. Indeed, this explanation has been combated by SCHWENDENER (1902). It appears to us, however, that the cohesion hypothesis has more to be said for it than any other of the numerous explanations hitherto given, but into the discussion of which we cannot enter (compare STEINBRINCK, Ber. d. bot. Gesell., 1898–1903). It has been shown also, both by KAMERLING (1898) and STEINBRINCK (1899 b), that cohesion and not imbibition may play an important part in many other phenomena of movement, such as those seen in the sporangia and elaters of Hepaticae, in water-tissues, and in the pappus of certain Compositae. Into the discussion of all these examples, however, we cannot enter here, but we must point out in conclusion that the cohesion of water of imbibition may possibly co-operate with processes connected with absorption, so that the contrast between these two sets of phenomena may not be so striking as would at first sight appear. Here we come once more to a point which we previously (p. 409) only hinted at. We saw that, according to BÜTSCHLI, a body capable of swelling has an alveolar structure, that the alveoli were filled with water when the body was in the swollen state, and that the walls of the alveoli collapsed when the body became desiccated. The forces which lead to the deformation of the alveolar walls we have now no difficulty in recognizing as those due to cohesion-tension in the evaporating liquid of the alveoli, and we might compare a single alveolus with the fibrous cell of the anther-wall.

Bibliography to Lecture XXXII.

ASKENASY. 1900. Verhandl. naturhist. Vereins Heidelberg, N.F. 6.
BÜTSCHLI. 1892. Über die mikroskop. Schäume u. das Protoplasma. Leipzig.
BÜTSCHLI. 1896. Abh. Kgl. Gesell. d. Wiss. Göttingen.
BÜTSCHLI. 1898. Unters. über Strukturen etc. Leipzig.
BÜTSCHLI. 1900. Zusammenfassender Bericht von Schuberg. Zool. Centrbl. 1900, 7.
DARWIN. 1876. Trans. Linn. Soc. II. Ser. 1, 149.
EICHHOLZ. 1885. Jahrb. f. wiss. Bot. 17, 543.
VAN GERICHTEN. 1876. Ber. d. chem. Gesell. 9, 1121.
HILDEBRAND. 1873. Jahrb. f. wiss. Bot. 9, 235.
KAMERLING. 1898. Botan. Centrbl. 73, 369.
KRAUS, GR. 1866. Jahrb. f. wiss. Bot. 5, 83.
NÄGELI, C. 1858. Pflanzenphys. Unters. 3. (Die Stärkekörner.)
NÄGELI and SCHWENDENER. 1877. Das Microskop, 2nd ed. Leipzig.
REINKE. 1879. Unters. über Quellung. (Hanstein's bot. Abhdlgn. 4.)
RODEWALD. 1895. Landw. Versuchsstationen, 45, 201.
SCHRODT. 1897. Ber. d. bot. Gesell. 15, 104.
SCHWENDENER. 1899. Sitzungsber. Berl. Akad. p. 101.
SCHWENDENER. 1902. Ibid. p. 1056.
STEINBRINCK. 1873. Untersuchungen über die anatomischen Ursachen des Aufspringens der Früchte. Diss. Bonn.
STEINBRINCK. 1878. Bot. Ztg. 36, 561.
STEINBRINCK. 1888. Ber. d. bot. Gesell. 6, 385.
STEINBRINCK. 1891. Flora, 74, 193.
STEINBRINCK. 1895. Dodonaea, Bot. Jaarboek, 7. Grundzüge d. Oeffnungsmechanik von Blütenstaub- u. a. Sporenbehältern.
STEINBRINCK. 1898. Ber. d. bot. Gesell. 16, 97.
STEINBRINCK. 1899 a. Festschr. f. Schwendener, p. 165. Berlin.
STEINBRINCK. 1899 b. Ber. d. bot. Gesell. 17, 170.
STEINBRINCK. 1903. Ibid. 21, 217.
VOLKENS. 1884. Jahrb. d. Berliner bot. Gartens, 3, 6.
ZIMMERMANN. 1881. Jahrb. f. wiss. Bot. 12, 542.

LECTURE XXXIII

MOVEMENTS DUE TO TURGOR AND GROWTH

So far as the movements referred to in the last lecture are concerned the presence or absence of protoplasm is of no consequence, for they take place just as well in dead as in living organs. As a rule, as soon as desiccation commences in a tissue the protoplasm dies, and it is only in plants which can endure complete desiccation that hygroscopic movements may be repeated again and again, the plant still remaining alive. This is true of most mosses, and, among higher plants, in *Selaginella lepidophylla*, which undergoes alterations in form very similar to those described as occurring in *Anastatica*. [The branches of many forest trees also exhibit movements of this character, often due to periodic alterations in the amount of water present in the cell-walls (GANONG, 1904).] We have now to study movements which are possible only in the *living* plant, movements which are not due to swelling or shrivelling of the cell-membranes nor yet to tension in these membranes induced by the evaporation of imbibition water. The causes of such movements, apart from locomotory movements (Lectures XLII and XLIII) lie rather in alterations in the cells, in which both the walls and their living contents participate equally—alterations which are conditioned either by osmotic pressure or growth in the cells, with both of which phenomena we have already made acquaintance. It will be necessary for us, however, to consider these phenomena somewhat further in detail.

We have already seen how osmotic pressure is brought about; we have also seen that in plasmolysis we have a method of determining this pressure, which has this great advantage that we do not need to know what the substances are which are present in the cell-sap and produce this pressure. All we have to do with here is the *amount* of osmotic pressure and how it *acts* on the cell-membrane. If we assume to start with that the osmotic pressure is insufficient to stretch the cell-wall we may also conclude that the plasmolytic solution is of the same concentration as the cell-sap. If a 2 per cent. solution of potassium nitrate produces plasmolysis then we may conclude that the cell-sap has the same osmotic value, although the cell-sap may consist of a mixture of all kinds of substances such as various sugars and organic acids, &c. Strictly speaking, the plasmolytic method gives us always rather too high a value, for if there be an obvious retraction of the protoplasm from the cell-wall, the plasmolysing liquid must have a somewhat higher value in terms of potassium nitrate than the sap. When we have estimated the concentration of the cell-sap in terms of potassium nitrate we can then calculate the osmotic pressure in the cell, since it is known that a 1 per cent. solution of potassium nitrate (=0·1 GM.) exerts a pressure of 3·5 atmospheres. With the aid of the table of isosmotic coefficients we are able to calculate the osmotic pressure value of any other solution we please. As a matter of fact, a potassium nitrate solution is peculiarly convenient for plasmolytic experiments, and very many investigations have been carried out with its aid. The following data with reference to the amount of osmotic pressure in different vegetable cells are taken from RYSSELBERGHE (1899, p. 23) :—

	Osmotic pressure in atmospheres.	Authority for the estimates given.
Peperomia (hypoderm of leaf)	3–4	WESTERMAIER
Plantago amplexicaule (peduncle)	6	DE VRIES
Phycomyces (hyphae)	7–8	LAURENT
Sorbus aucuparia	9	DE VRIES
Foeniculum (peduncle)	9–12	AMBRONN
Helianthus (medulla)	13	DE VRIES

	Osmotic pressures in atmospheres.	Authority for the estimates given.
Phaseolus (pulvinus)	10–12	HILBURG
Pinus (cambium)	13–16	WIELER
Populus (cambium)	14–15	,,
Pinus sylvestris (medullary rays)	13–21	,,
Pinus nigra ,, ,,	16–21	,,

Speaking generally, we may say then that in ordinary plant-cells the osmotic pressure is equivalent to from five to ten atmospheres, but that variations both above and below this average are not infrequent. The pressure in ordinary parenchymatous cells, if they be in an extremely starved condition (STANGE, 1892, 391), does not fall below 3·5 atmospheres, and even in the cells of leaves which have fallen off and are becoming yellow a quite obvious osmotic pressure may still always be recognized. Whether or not a far less pressure occurs in the tubers of artichoke than in other cells, as H. FISCHER (1898) and COPELAND (1896) affirm, requires further confirmation. Examples of great osmotic pressure, in addition to those quoted at the end of the table given above, are to be found in the onion and beet, which possess large reserves of grape and cane sugar, and in which pressures of fifteen to twenty-one atmospheres have been registered, but the maximum pressure has been recorded in the nodal cells of grasses, where PFEFFER has observed (1893,399) an osmotic pressure amounting to as much as forty atmospheres. Greater pressures even than these, which occur only under certain conditions, will be referred to afterwards.

It has already been pointed out that the osmotic pressure in a cell never remains constant ; continual variations or adaptations are for ever taking place in it. When the cell grows the absorption of water leads to a reduction in the concentration of the cell-sap, and consequently to a reduction in osmotic pressure, but if such a reduced pressure does not make its appearance, or appearing does not continue, it may be assumed that a re-formation of osmotic substance has taken place which rapidly leads to the re-establishment of the pressure previously existing. Far more remarkable adaptations are obtainable by cultivating the cells in concentrated media, for we have already seen that the pressure rapidly rises and may reach the enormous pressure of 150 atmospheres. An internal pressure as great as that is, of course, possible only if the external liquid be capable of exerting a strong osmotic pressure ; for instance, if we put a cell which has been lying in a highly concentrated sugar solution into water the internal pressure may operate unilaterally and be sufficiently great to burst the cell. Such ruptures of cells, as we shall presently see, occur normally in some cases during development, but, generally speaking, osmotic pressure is regulated in such a way that the cell-wall is stretched only up to its limits of elasticity.

Let us now glance at the significance of osmotic pressure. In many cases, e. g. in the beet and onion, great osmotic pressure is to be regarded as a secondary and undesirable result of the accumulation of large quantities of reserves, so much so that in most reserve stores an effort is made, by changing these bodies into others which are insoluble and which have large molecules (e. g. starch), to reduce the osmotic activity of the cell-sap. In other cases it is quite likely that such high osmotic pressures are of some service to the plant. Apart altogether from the fact that, generally speaking, osmotic pressure would appear to be favourable to growth, one important function must be ascribed to it, viz. that young cells attain by its means alone the necessary degree of rigidity. In general, the rigidity of the elements which specially subserve mechanical ends is attained by employing firm cell-walls, but CORRENS (1891) has shown that in specific mechanical tissues osmotic pressure may play an important part, as in the hairs of *Aristolochia*, whose articulating cells are thin-walled and maintain the necessary rigidity only by marked turgor pressure (twenty-two atmospheres).

It is easily understood how far osmotic pressure has an effect on the rigidity of the cell-wall. The pressure *stretches* the delicate cell-membrane until its elasticity equals the pressure, then the wall resists all further attempts at deformation, and as a result the cell becomes more rigid. Increase in rigidity owing to stretching is well exemplified by the behaviour of a thin-walled caoutchouc balloon, which, when blown out, maintains a constant shape but which without such extension of its wall is by no means firm. Osmotic pressure as an agent in the production of rigidity is, however, but little employed in the plant; it occurs in the lower forms which live in water or in moist air, but in the higher plant only in young parts still capable of growth. Later on, secondary thickening in the cell-wall takes on the duty of maintaining rigidity; such secondary thickening would be distinctly disadvantageous in vigorously growing organs. Certainly dependence on osmotic pressure for the maintenance of rigidity in such organs has its dangers, for on a warm summer's day they become limp, that is to say their rigidity has been destroyed by excessive evaporation.

It is of importance for us now to know *to what extent* the cell-wall may be osmotically stretched. This is determined by the amount of *contraction* that takes place when turgor is neutralized. Turgor may be arrested by wilting, by killing the cells in hot water, or by *plasmolysing* them. It will then be seen that all *growing* cell-walls are markedly stretched, so much so that, as a rule, a contraction in length from 3 per cent. to 20 per cent. and about 10 per cent. reduction in diameter take place when turgor is abolished (De Vries, 1877; Schwendener and Krabbe, 1898). If we stretch the walls of plasmolysed cells by means of a weight until they have attained the same length that they had in the turgid condition we are obviously able to determine the amount of the osmotic pressure in the cells, apart from the values of the osmotic activity of cane sugar, obtained by experimental apparatus, and apart from the use of the plasmolytic method.

In full-grown cells the extensibility of the cell-wall is so limited that an observable contraction after plasmolysis scarcely exists. Exceptional cases are known, however, for fully grown cells exist which exhibit highly extensible walls. Such cells occur in the leaf articulations, and we shall see later what an important part they play in the movements of many leaves. At present it need only be noted that such cells occur also in the stamens of such plants as the Cynareae. Indeed, these cells are among the most extensible known in the whole vegetable kingdom, for Pfeffer (1892, p. 234) found that when plasmolysed they contracted to *half* their original length; only in the seeds of *Haemanthus* perhaps have cells with still more extensible cell-walls been observed (Hildebrand, 1900).

The determination of osmotic pressure, it may be said here, cannot be carried out offhand by the plasmolytic method in cells which exhibit as great extensibility as those of the Cynareae do. Plasmolysis indicates to us, indeed, the occurrence of osmotic pressure in the cell that has contracted to *half the length* it was in the turgescent condition, and in which consequently there is relatively twice as much osmotic material as at first. The value for the osmotic pressure so obtained must be in this case estimated at one half of that. It would appear also that in cases where the contractions amount only to 10–20 per cent. of the original lengths, corresponding corrections must be made on values determined by plasmolysis, corrections which can only be reached by exact calculation of the decrease in the cell *volume* in each case.

From the action of osmotic pressure on the cell-wall just described there arises the possibility of movements of the cell. Looking first of all at a single cell we can see that a simple elongation, that is to say a rectilinear movement, will take place either when osmotic pressure increases, or when the wall becomes more extensible. Similarly, reduction in osmotic pressure, accompanied by contraction of the cell-wall, will also lead to shortening in a rectilinear direction.

If, however, the cell-wall be not of the same consistence all round, then an alteration in osmotic pressure will always lead to an alteration in form. The best known case of this kind is the movements of guard-cells, of which we have already given an account. A glance at Fig. 8 (p. 39) will remind us that in the guard-cell the convex side is thinner, and, therefore, more extensible than the concave side. As osmotic pressure increases the curvature of the cell already existing also increases, and it is easily seen that, by the appropriate distribution of more resistant areas in the wall, a cylindrical cell may be made to exhibit not only simple curvature but torsion and twining as well, such as are seen in Fig. 119 (p. 406). In nature, however, such torsions and twinings are due always to phenomena of growth and scarcely to osmotic pressure.

Movements arising from variations in turgidity occur much more frequently in multicellular tissues than in single cells. Inasmuch as in these cases the individual cells are osmotically *unequally* stretched there arise widespread tissue tensions such as those referred to in Lect. XXIII (p. 297). Tissue tensions were referable, as we found, to unequal degrees of growth in the separate components of these tissues, but it is obvious that the only condition necessary for tissue tension is the *unequal efforts to elongate* of the different parts, and, further, that it is immaterial whether that elongation be effected by osmotic pressure, growth, or some other factor. The example we took on that occasion was a stem or similar structure, whose central region had greater powers of extension than the periphery; as a consequence, we found that the peripheral regions were in a state of tension, while the medulla was in a state of compression, and that the total length of the organ was the resultant of these opposing factors.

So long as these antagonistic parts are distributed as they are in a normal growing stem any alteration in the turgor conditions can only result in an alteration in the length of the entire organ, and cannot induce any curvature, torsion, &c. The significance of these tensions, which are of common occurrence, must be purely mechanical; for just as the single cell is rendered rigid by osmotic pressure so a stem acquires rigidity from *tissue tensions*.

In the typical stem, &c., we find that the tissues which contract are uniformly distributed all round the compressed central cylinder, but as soon as that *uniformity* of distribution is interfered with curvatures at once take place. Such disturbances are of frequent occurrence in nature, according as one of the longitudinal halves of the organ under consideration gains or loses in turgidity. Experimentally, it is perfectly easy to demonstrate the curvature resulting from tissue tensions; all one need do is to split a growing shoot-axis longitudinally, when the medulla will thereby be enabled to extend itself and will become convex, while the cortex in its efforts to contract will become concave.

Movements due to variations in turgor are frequently *reversible* if the *factors* be also reversible, for the cell-walls are both extensible and *elastic*. Movements of this kind are known as *variation movements* and stand out in contrast to *growth* or *nutation movements*. These latter movements also start with stretching of the cell-walls, and hence may, in their earlier stages, be reversed by plasmolysis, but after a short time the osmotically extended membrane undergoes *growth* and its elongation and the *movement* itself become *permanent*. Growth movements, like those due to turgor, may be rectilinear, curved, or spiral, &c. It is unnecessary to consider this in further detail, for the analogy with variation movements is complete. (As to growth itself, see Lect. XXI.) A few remarks of a more general character may, however, be added here.

In all movements, whether they be due to growth or turgor, a certain amount of energy must be expended in the overcoming of external and internal resistances. With internal resistances we are but slightly acquainted, but as to the external, the elaborate experimental researches of PFEFFER (1893) have given us very full particulars. These resistances may be very slight if the plant grows

in a straight line in water or air, but they reach high values when, for example, a root attempts to force its way into the ground or encounters stones in its passage through the soil. A considerable amount of work must often be accomplished when curvatures take place, and the amount is all the greater the nearer to the base of the organ the zone of curvature lies, for the weight of the passive erect portion of the plant is so much the greater.

It would appear that the energy for carrying out these variation movements can be obtained only from osmotic pressure, but since, as we previously saw, far greater forces are developed in surface growth of the cell-wall by excretion-energy than by osmotic pressure, we may assume that excretion-energy also plays an important part in these external activities of the growing plant. According to PFEFFER's researches, although these external activities are to be referred *exclusively* to turgor pressure, the plant is able, however, to employ the *whole* of that pressure in the overcoming of resistance.

Without going into detail, we may merely note that PFEFFER imbedded the part of the plant under investigation in plaster of Paris, forming a general resistant layer, and, with the aid of appropriate apparatus, was able to measure the pressure which the plant exerted to overcome the resistance thus given to its expansion. External pressures of this kind often reached as much as twelve atmospheres.

So long as the cell encounters no *external* obstacle to its expansion the whole of the osmotic energy is devoted to the stretching of the cell-wall, but after enclosure in plaster of Paris the wall is extended by *growth*, and, as the extension progresses, the osmotic pressure is directed against the obstacle. When extension is complete the whole internal pressure may in the long run be devoted to the performance of external work, and in many cases, as the resistance increases, not only does the extension of the cell-wall increase up to its limits but the osmotic pressure also rises above the normal.

The first series of movements we have to study are those which are illustrated by ripe fruits and spores, and which may be termed ejaculatory movements. All of these are characterized by their suddenness, and that suddenness is rendered possible only by tensions induced between tissues, cells, or parts of a cell, and which are equalized in a moment. A phenomenon such as this we have already recognized as taking place in hygroscopic movements, where, in addition to the slow oscillation due to gradual absorption and evaporation of water, irregular movements also occur. At the moment when the dehiscence of a capsule is effected by a sudden adjustment of tensions, not infrequently parts of the fruit wall or the seeds are thereby thrown off. The ejaculatory movements we have yet to study may be compared, from a biological point of view, with these movements in dry fruits. So far as the mechanism is concerned they are closely related to them, but the difference lies in this, that the tensions previously mentioned are conditioned by the swelling of the membrane or cohesion of imbibition water, while those we have now to speak of are due to osmotic pressure.

Let us commence with cases where the tensions are those which occur in single cells, as, for example, in the spore cases (asci) of the Ascomycetes (DE BARY, 1884), and let us select *Ascobolus* as our first example. The asci are elongated cells, each containing eight young cells or spores in addition to the normal cell constituents, viz. a peripheral protoplasmic layer—much reduced in amount—and an osmotically active cell-sap. The asci are aggregated in thousands into a single layer (hymenium), and are mixed with narrow sterile cells (paraphyses). When the ascus becomes ripe a marked increase in the osmotic pressure occurs, along with a subsequent increase in volume, which may easily result in the doubling of the original length and diameter of the ascus. If the ascus is cut off or plasmolysed it regains its original size, so that the increase in size is not due to growth. At a certain moment in the natural course of develop-

ment a circular patch at the apex of the ascus can no longer withstand the pressure inside, and it forthwith bursts. In consequence of the pressure of the elastic and contractile membrane the entire contents of the cells are at once ejected through the opening, in the case of *Ascobolus* to a height of seven or more cm., and in *Sordaria fimiseda* to as much as 15 cm., while the wall at once contracts to its original dimensions. In *Ascobolus* a large number of asci burst at the same moment in the day, i. e. between one and three o'clock, the extension and extrusion from the hymenial surface having begun the evening before. The ejection occurs then after a very gentle shaking, which probably acts so as to cause a slight bending of the ascus and in this way an increased stretching of the membrane beyond the capacity of the apical region to withstand; at the same time it can scarcely be doubted that in the long run ejection may occur without such oscillation. This daily periodicity in the ejection of spores is obviously dependent on light, but into these relationships we cannot enter here. Nor can we discuss in detail the various means whereby asci open, for the isolation of an apical lid as in *Ascobolus* is by no means general.

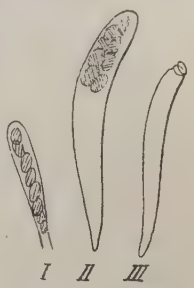


Fig. 126. *Ascobolus furfuraceus*. *I*, young ascus. *II*, ripe ascus. *III*, empty ascus. × 150. After DE BARY (Morph. d. Pilze. Leipzig, 1884).

A certain amount of interest, however, attaches to the type of ascus which ejects its spores one at a time, as takes place in many Pyrenomycetes, e. g. *Sphaeria scirpi* (PRINGSHEIM, 1858). Before the spores begin to be ejected a sudden extension of the ascus takes place, amounting to as much as three times its original length. As a consequence, the outer lamella of the wall of the ascus is ruptured, and rolls up, while the inner lamella becomes stretched (Fig. 127); a tension thus arises between two layers of the cell-wall not previously exhibiting any difference. By the vigorous extension of the ascus the spores (which are in this case multicellular), are pushed towards the apex, and very soon the uppermost spore is ejected through the apical opening. As the ascus contracts it becomes slightly shorter and the next spore at the same time closes the aperture, and so renders any further exit of cell contents impossible. Thereupon ensues a fresh increase in osmotic tension in the ascus, increasing until the second spore is ejected through the narrow aperture, and so on. When all the spores are shed the ascus contracts markedly, and at the same time its wall becomes much swollen, and hence it is very apparent that the ejection is not occasioned by osmotic pressure, or at least not by that only, but that swelling of the cell-membrane, may take part in the process. Similarly in many other lower organisms the spores are pressed out of the mother-cell by swelling of certain parts of the cell-wall.

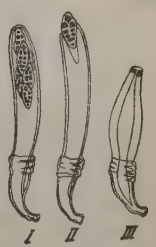


Fig 127. *Sphaeria scirpi*. *I*, extended ascus, the outer layer of the wall burst. *II*, the last spore has not as yet been extruded. *III*, empty ascus. After PFEFFER (Pflanzenphysiologie, 1st edition).

Osmotic activity is, however, responsible for the ejection of the spores of *Empusa* and its allies (e. g. *Basidiobolus*, p. 249) and for the corresponding phenomenon in the sporangia of *Pilobolus*. Let us consider the case of *Pilobolus crystallinus* (compare DE BARY, 1884). In this plant we have a sporangiophore (*t*, in Fig. 128) which is much swollen and whose termination is inserted into the base of the sporangium. When the pressure in the sporangiophore has reached a certain height a circular rupture occurs in the membrane at *r* and the contents of the cell are, owing to the contraction of the wall, ejected just as in the case of the ascus of *Ascobolus*. The liquid which is ejected from the sporangium in this case may be thrown into the air to a height of a metre.

There is another series of examples which may serve as an illustration of ejaculatory movements, which are due not to the activity of a single cell but to tensions in many. The method met with in the squirting cucumber (*Ecballium elaterium*) (HILDEBRAND, 1873) reminds one of the phenomena of ejection as illustrated by *Ascobolus*. The elongated ovoid fruit (Fig. 129), owing to the bending of the peduncle, turns its base upward. It consists of a wall formed of several layers of cells, enclosing slimy contents enveloping the seeds. When the fruit is ripe, as is indicated by its turning yellow, the part of the peduncle nearest to the fruit wall becomes loose, and if the fruit be lightly touched it springs out of the fruit-wall just like the cork out of a champagne bottle (Fig. 129, *II*). At the same moment the mucilaginous contents of the fruit, together with the seeds, are ejected with great force to a considerable distance. It is obvious that the fruit-wall contracts during this process and the amount of contraction may readily be measured. Thus a contraction in length from 100 to 86, and in diameter from 100 to 84, has been observed in a fruit not perfectly ripe, and in all probability a still greater contraction occurs in the absolutely ripe condition, but owing to the readiness with which such fruits explode it is by no means easy to carry out measurements on them. The fruit-wall must, therefore, have been in a stretched condition previous to the bursting. Researches hitherto made on *Ecballium* have not determined whether this stretching, as in the case of *Ascobolus*, is due to osmotic pressure or to pressure resulting from the swelling of the contents. On the contrary it appears to us improbable that the pressure of the fruit wall itself operates in the way HILDEBRAND suggests. According to this author the external layers of the wall, which are composed of large, succulent, thin-walled cells, are able to extend more than the inner layers, and must in consequence exert pressure on the interior of the fruit and so bring about the explosion. As a matter of fact, however, the layers of the fruit-wall including the *outer* ones, as DUTROCHET (1837) long ago observed, *shorten* during the contraction of the fruit-wall.

Fig. 128. *Pilobolus*, Diagrammatic longitudinal section. *t*, upper end of the sporangiophore; *r*, line of rupture; *sp*, sporangium. After DE BARY (1884).

In the majority of cases, certainly, stretching is induced in the fruit-wall in the way in which HILDEBRAND believed it takes place in *Ecballium*, that is, by differential stretching of different cell layers, by tissue expansion. As an example of this we may take the case of *Impatiens* (EICHHOLZ, 1885). The fruit is composed of five carpels, and the seeds arise from an axile placenta. When the fruit is ripe the five thin lateral walls separate, not only from the placenta but also from the outer wall, and this latter splits into five valves, each corresponding to the region between two lateral walls. When fully ripe the least touch is sufficient to isolate the five valves from each other, and each of these rolls itself up suddenly, like a watch-spring, beginning at the base, striking against the seeds, and throwing them out. If one attempts to bend the valve straight again, it breaks across, but if it be plasmolysed all opposition to the straightening is removed. Hence it may be concluded that we are dealing here with an osmotic phenomenon, and closer investigation demonstrates that a layer of parenchyma lying under the external epidermis acts dynamically, swelling, and to it the internal cell mass acts antagonistically. In the complete fruit, this layer is positively stretched, it endeavours to expand, and this at once results in the isolation of the valves, because its cells possess very

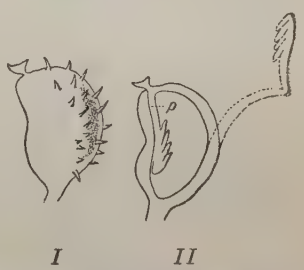
Fig. 129. Fruit of *Ecballium Elaterium*. *I*, in longitudinal section. *II*, open at the top. After HILDEBRAND (1873).

elastic walls and present a very high osmotic pressure (7·5 atmospheres). Whether the inner layers of the wall exhibit equal osmotic pressure but less extensible membranes, or whether their osmotic pressure is less, does not appear to have been determined; a difference in the extensibility of the membranes is quite sufficient, however, to account for the result.

Cyclanthera pedata (HILDEBRAND, 1873) may be compared in all respects with *Impatiens* so far as its mechanism is concerned, but it differs entirely in its form. The fruit of this plant consists of three carpels, forming a placenta, however, on only *one* of the sutures, bearing two rows of obliquely-placed seeds. The position of the placenta in the interior may readily be recognized in the asymmetrical fruit (Fig. 130, *I*), for on this side there is less bulging, and the spines, developed on the other side, are here absent.

When the fruit is ripe the wall bursts open, from the top downwards, into two longitudinal halves, which curve outwards, so that the exterior of both becomes concave, in consequence of tissue tensions which, however, operate in the reverse way to those in *Impatiens*. Certain peculiar arrangements in the interior of the fruit facilitate the ejection of the seeds, for the placenta becomes released from that half of the fruit with which it was originally united, but remains firmly attached to the apex of the other curving half, and hence, when the sudden rupture occurs, it is slung backwards, and the seeds are in this way released from their attachment and jerked with considerable force into the air.

Similar tensions, leading to movements, are found not merely in fruits but in other regions of the plant, and are especially frequent in flowers. The relation of the stamens of many Leguminosae (e.g. *Spartium*) to the carina is the only case that need be referred to; these are suddenly released when the flower is visited by an insect, whereby the pollen is ejected. These phenomena do not appear to have been investigated from the physiological standpoint, so that we need not discuss them further. On the other hand, the ejaculatory movements of the stamens of the Urticaceae have been accurately studied, and a brief reference may be made to them. Each stamen when the flower opens is curved inwards so that the anther comes to touch the base of the filament. The concave side of the filament is thus in a state of compression and attempts to straighten itself, but cannot do so on account of certain obstacles. At first sight it would appear as if the perianth on the one hand, and ovary on the other, between which the anthers are pinched, were the cause, but, as ASKENASY (1879) showed, it is possible to remove a stamen from the flower without any consequent straightening of the filament. The anther is glued to the base of the filament and it is only when this resistance is overcome that the concave side straightens itself, while the anther opens with a sudden jerk, at the same time ejecting the pollen. A touch or a slight heating may accelerate this movement, but it takes place automatically when the osmotic pressure has become sufficiently great. If the filaments be plasmolysed the tension is abolished, and hence we may conclude that osmotic pressure alone is responsible for the movement. The plasmolysed filaments, however, present a certain resistance to attempts to straighten them on account of the fact that the convex sides are more fully grown than the concave sides; the rapidity of the releasing movement must therefore overcome the resistance offered by the greater length of the convex side.



I *II*

Fig. 130. Fruit of *Cyclanthera explodens*. *I*, general view. *II*, longitudinal section showing manner of opening; *p*, placenta. After HILDEBRAND (1873).

In the discussion of these examples of slinging movements it has been again

and again remarked that equalizing of tensions is generally brought about after the application of a stimulus, such as contact, shaking, &c., but that they may take place to all appearance of their own accord when the tension has reached a certain amount. To prove this is, however, by no means easy, for, obviously, the nearer the organ approaches to a condition of ripeness, shocks, ever decreasing in intensity, are sufficient for the purpose, the application of which are scarcely avoidable in the course of the observation. We have yet to discuss the significance of these shocks.

In the preceding pages we have often spoken of such shocks, and have seen that very frequently, by their means, energies stored up in the organisms are released; they act as 'stimuli.' In speaking of plant formation, we saw that contact especially might act in this way, and in Lect. XXXVIII we shall learn to recognize a whole series of movements which were initiated by contact. The question now before us is, must we group such slinging movements as we have just been discussing alongside the movements resulting from the application of stimuli, such as we have yet to consider, or are they to be placed in another category, viz. 'autonomous' movements?

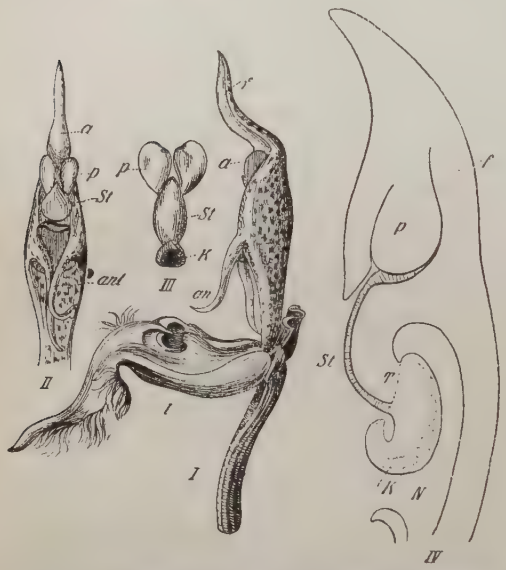


Fig. 131. *Catasetum saccatum*. *I*, the flower after removal of five of the perianth leaves. *II*, front view of the column. *III*, pollinium and cement disc. *I–III*, after DARWIN. *IV*, longitudinal section of the column (from nature). *a*, anther; *an*, antenna; *f*, filament; *K*, cement disc; *St*, caudicle; *p*, pollinium; *T*, boundary between the cement disc and rostellum; *N*, stigmatic cavity; *l*, labellum.

By autonomous movements we mean such as are induced by some internal factor and not the result of the application of an external stimulus (Lect. XLI). From what has been said it is evident that the ejaculatory movements manifested by spores and succulent fruits may be *autonomous*. When such movements follow a blow or shaking it is obvious that the immediate result consists only in a local increase in tension which would arise spontaneously in the course of further ripening. Seeing that the blow does not provide the *force* which brings about the ejection we have here to deal with a *releasing* force only, or a stimulus. This stimulus differs essentially from those usually met with in the plant, so that it is doubtful whether the ejaculatory movements are to be counted as stimulus movements or not. There are, however, slinging movements which are undoubtedly typical *stimulus-movements*, and in order to show clearly the difference between these two types it will be advisable to discuss one example.

The slinging movement in question is exemplified in the flower of the orchid *Catasetum*, the structure of which will be made clear by a study of Fig. 131 (DARWIN, 1877). In *I* the entire flower is shown after removal of five of the perianth leaves; the one left is the large labellum (*l*). In the middle stands the column, from the face of which project two horn-like appendages, the so-called antennae (*an*). Looking at the column in face view (*II*) it will be seen that the antennae arise at the base of the massive anther (*a*). As in all

orchids the pollen-grains are here united into pollinia, and the pollinia are connected by means of a stalk, the caudicle (*st*), with a cement disc (*K*). The space relations of these three bodies may be best seen by an examination of the longitudinal section of the column (Fig. 131, *IV*). The anther, it will be seen, placed at the end of the long filament (*f*) is curved downwards, and it may be noted that the caudicle uniting the pollinium and the cement disc is bent over a small cushion of tissue, the rostellum; perhaps it would be more accurate to say that the caudicle is composed of the outermost cells of the rostellum and has become differentiated from the deeper seated parts. The cement disc is also a part of the rostellum, and only that side of it which faces backward is sticky.

If the flower be placed under appropriate external conditions, one of these especially being a suitable temperature, and if one of the antennae be lightly touched with a pencil or splinter of wood, the caudicle and cement disc are released from the rostellum, the caudicle straightens itself, and throws the cement disc forwards with considerable force. The movement is so vigorous that the entire pollinium is jerked out of the flower and is shot through the air, cement disc first. If it meets any obstacle in its passage the cement disc becomes firmly attached to it. The biological significance of the movement is perfectly clear, it is one of the extremely interesting adaptations one meets with in Orchidaceae for bringing about cross-pollination by insect agency. It is unnecessary to go into biological details; what more immediately concerns us is, in the first place, the nature of the tensions in the caudicle, which is obviously the mechanical cause of the movement and, further, the significance of the friction applied to the antennae.

As to the nature of the tensions in the caudicle there have been no investigations, and absolutely nothing is known as to how the effort to elongate the inside of the caudicle arises; it may be due to swelling, osmotic pressure, or growth. An investigation of the structure of the cells of the caudicle should prove an acceptable subject for research at the hands of the physiological anatomist. Moreover, the straightening of the caudicle longitudinally is by no means the only movement which takes place; at the same time, an inrolling of its edges occur, so that the outer parts along the edge of the caudicle exhibit stronger efforts to elongate than the inner parts.

The stipe may be made to spring loose not only by rubbing the antennae but also by the application of pressure to the caudicle itself. Such a pressure may be compared with the shaking or bending in the slinging movements previously mentioned; obviously it leads to increased tension and so to an explosion when the resistance is overcome. The touching of the antennae is another matter altogether; there we have to deal with a genuine stimulus, and this stimulus is applied at a considerable distance from the place where the activity is manifested. It is quite out of the question that any contact between a pencil point and the antenna can possibly lead to a mechanical deformation of the caudicle, and so to an increased tension in that organ. What the precedent phenomena are, how they are transmitted, and how they lead to an ejection of the pollinia is as yet quite unknown.

Hitherto we have considered only such movements as take place in response to an external stimulus applied to some part of the living plant. This stimulus may be light, heat, electricity, gravity, or a mechanical or chemical action of some body. We may indeed distinguish two types of action of such stimuli, viz. general and special. The *general* stimuli or so-called *formal conditions* are necessary in order that the plant may, first of all, be in the condition to react, in order that growth and movement may be possible, and in order that *special* stimuli may be able to induce movements in it. To the category of general stimuli belong a certain degree of temperature, a certain amount of oxygen, and all substances which we have termed nutrients. The special stimuli very fre-

quently either affect different *parts* of the plants with different intensity or the intensity itself alters from *time to time.* Every stimulus, as we have already sufficiently shown, is a *releasing* stimulus only. That the formal conditions (general vital conditions) operate also as stimuli has not always been clearly appreciated ; but in the majority of cases it is impossible to regard them in any other light, and it is often, indeed, extremely difficult to differentiate them from *special* stimuli.

Given the general conditions the special stimuli lead to a number of *internal* processes which we shall get to know about more exactly later on, and, finally, to movement ; this we may term the *stimulus* reaction, or, more accurately, the *visible final reaction.* We say that the stimulus *induces* a certain movement ; the movement is therefore known as an *induced* or *paratonic* movement. Movements, which are outwardly indistinguishable from paratonic movements, are also frequently to be met with, which are *not* so induced ; these we speak of as *autonomous* movements.

We must now attempt to formulate some suitable classification of stimulus movements. We might group them either according to the nature of the *stimulus*, that is to say, movements induced by heat, light, and so on, or we may base it on the nature of the *reaction*, or, finally, on the *biological significance* of the movement. We will select the nature of the *reaction* as the principle to follow, and distinguish first of all the reactions exhibited by *motile organisms*, which we shall discuss in the two final lectures, as opposed to the reactions manifested by *fixed forms.* The latter may represent either alteration in length, or bendings, twistings, or twinings, as illustrated on p. 406 in Fig. 119. Owing to these alterations in form a part at least of the organ takes up a new relationship to others, or occupies a new situation. When the new situation shows a relation to the *direction* of application of the *stimulus* we speak of movement as a *tropism.* When, however, the stimulus is not applied in any definite direction, or when the orientation of the organ shows no relation to it but is determined by the activity of the plant itself, we speak of the movements as *nastic.* We will begin by considering *directive movements* or *tropisms*, and then deal with bending or *nastic movements*, endeavouring in each case to determine whether they are due to *growth* or to *turgor.*

Bibliography to Lecture XXXIII.

Askenasy. 1879. Verhandl. naturw. Vereins Heidelberg, N. p. 2.
De Bary. 1884. Morphologie u. Biologie d. Pilze etc. Leipzig.
Copeland. 1896. Einfl. d. Temperatur u. des Lichtes auf d. Turgor. Diss. Halle.
Correns. 1891. Jahrb. f. wiss. Bot. 22, 161.
Darwin. 1877. Die Befruchtung d. Orchideen.
Dutrochet. 1837. Mém. pour servir à l'histoire des végétaux etc. Paris. 1, 451.
Eichholz. 1885. Jahrb. f. wiss. Bot. 17, 543.
Fischer, H. 1898. Cohn's Beitr. z. Biologie, 8, 53.
[Ganong. 1904. Annals of Botany, 18, 631 (comp. Bot. Ztg. 1905).]
Hildebrand. 1873. Jahrb. f. wiss. Bot. 9, 235.
Hildebrand. 1900. Ber. d. bot. Gesell. 18, 376.
Pfeffer. 1892. Energetik. (Abh. K. Gesell. d. Wiss. Leipzig, 18.)
Pfeffer. 1893. Druck- u. Arbeitsleistung. (Ibid. 20.)
Pringsheim. 1858. Jahrb. f. wiss. Bot. 1, 189.
Rysselberghe. 1899. Mém. couron. Acad. belg. in-8°, 28, 1.
Schwendener and Krabbe. 1893. Jahrb. f. wiss. Bot. 25, 323.
Stange. 1892. Bot. Ztg. 50, 253.
De Vries. 1877. Unters. über die mech. Ursachen d. Zellstreckung. Leipzig.

LECTURE XXXIV

GEOTROPISM. I

No special botanical knowledge is required to convince oneself of the fact, which all practical experience teaches us, that plant organs assume certain definite positions in space. The tree trunks in a fir-wood all stand perfectly erect and are hence all parallel to each other ; their branches, large and small, always conform to rule, still their lie cannot be stated only in terms of the angle which they make with the perpendicular, since that obviously does not comprise all the relations they bear to the chief axis for the time being. Instead of a fir-tree let us examine a seedling, thus simplifying the problem, since, in this latter case, the only organs present, at least at first, are those which grow perpendicularly. At the same time we can observe here, much more readily than in the case of a tree, the totally different behaviour of the root and the stem. Both grow perpendicularly to the earth's surface, but the stem grows upwards, while the root grows downwards. If we place the seedling in an unnatural position, e.g. horizontally, we note immediately that both organs begin to bend, the root downwards, the plumule upwards. Since these curvatures take place, not at the place where stem and root meet, but near the apices of both organs, a varying length of axis remains horizontal, and only the two terminations resume the perpendicular position on bending, growth being continued in that direction. Since almost every organ in the plant has a certain definite position of rest and endeavours to regain it after it has been interfered with, we must grant to the plant the capacity of orientating itself in space, and the movements which its members exhibit in their endeavours to assume their natural and appropriate positions, not by simple bending merely but also by torsions and twinings, we term *movements of orientation*. Obviously this orientation is the result of the action of certain external factors such as the distribution of light, water, &c., and the plant must possess sense organs of some kind by means of which it appreciates the influences thus brought to bear upon it by the environment.

Very frequently the orientation of an organ is dependent on the combined influence of several factors, but in the simple cases with which we will begin, as the downward curving of the root and the upward curving of the shoot, apparently one agent only is concerned, viz. *gravity*. That gravity is directly responsible for the perpendicular mode of growth of root and shoot may be shown by direct observation, for these organs are orientated in the same way over the whole surface of the globe, that is, parallel to the earth's radii, and we know of no other force which acts universally in this direction. Still it is not on reflections such as these but on the experiments of KNIGHT (1806), and SACHS (1874) that our knowledge of the subject is actually founded. KNIGHT'S experiments rest on the following basis :—Obviously gravity can cause the root to grow down and the shoot to grow up only if the seed remain in a state of rest and in the same relative position with reference to the direction of the earth's attraction ; hence KNIGHT concluded that if the lie of the germinating seed were continuously and rapidly changed by being subjected to the influence of another, say centrifugal force, the effect of gravity might be suspended.

He, therefore, fastened a number of germinating seeds to the rim of a wheel in a variety of positions, so that the protruding radicles pointed outwards, inwards, and tangentially, and rotated the wheel on a horizontal axis. As the wheel was made to revolve at a very considerable speed, not only was the unilateral influence of gravity neutralized, but at the same time a very consider-

able centrifugal force was exerted which on its part affected the seedlings on one side only.

'I soon had the satisfaction of seeing,' writes Knight, 'that the roots, in whatever direction they stood in reference to the position of the seed, turned their apices outwards from the rim of the wheel and in later growth formed nearly a right angle with the axle. The young stems on the other hand grew in the opposite direction, and in a few days all their apices met in the centre of the wheel.'

In this experiment the seedlings are influenced by centrifugal force exactly in the same way as they are by gravity when grown under natural conditions.

In another experiment Knight allowed gravity and centrifugal force to act at the *same time* but in *different directions*. The seedlings were fastened to a horizontally rotating disc and the distance of the plants from the centre and the speed of rotation of the disc were so arranged that the mechanical effect of gravity and of the centrifugal force were equal. Under such circumstances the roots grow outwards and downwards at angle of 45°, while the stem grew upwards and inwards at a similar angle. When the speed of

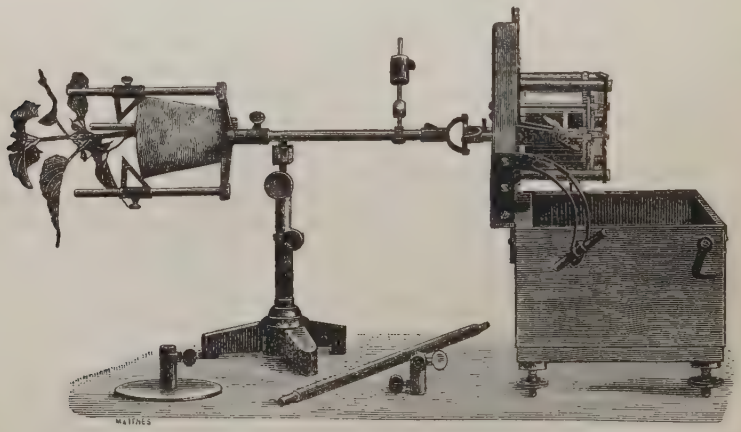


Fig. 132. Pfeffer's Klinostat, as manufactured by Albrecht of Tübingen.

rotation was increased, the axes of the seedlings took up a position which gradually approached the horizontal. From this it must be concluded that the plant is unable to discriminate between centrifugal force and gravity and that one force may be replaced by the other. Both forces have this in common, however, that they act as accelerating forces on the plant body.

Long afterwards, Sachs's experiments (1874) added very important facts to the fundamental data established by Knight. In Sachs's as in Knight's first experiments the seedlings were made to revolve round a *horizontal* axis but the speed of the revolution was very low, viz. about one revolution in 10–20 minutes. This speed indeed is so low that no centrifugal force worth mentioning is produced; since, however, the unilateral action of gravity is eliminated owing to the continuous revolving movement of the disc, the roots and shoots go on growing in the directions in which they were originally orientated. By the employment of this apparatus, the curving (κλίνειν) of the plant is inhibited, so that Sachs (1879) termed it a *Klinostat*. Fig. 132 illustrates such an apparatus in operation. The horizontal axis is driven by clockwork and to this axis the plant is attached; to the mechanical arrangements for altering the speed of rotation we need not pay any attention.

The movements of orientation of the plant we term ' tropic ' curvatures, and the capacity it possesses for producing such curvatures we term ' tropism ' (p. 428). According to the nature of the external cause we may distinguish tropic movements due to gravity, light, &c., or, in other words, we may speak of geotropism, phototropism, &c. In the present lecture we have to deal with *geotropism*, and from what has been said it will be apparent that we may recognize two varieties of geotropism : *positive* geotropism—such as are exhibited by roots and all other organs whose direction of growth is towards the earth's centre, and *negative* geotropism as manifested by shoots and such other parts of the plant as grow away from the earth's centre, parallel to a radius from it. Although root and shoot may be considered as characteristic organs illustrating the two types of geotropism, it would be quite incorrect to assume that the geotropic reaction was determined by the morphological nature of the organ. The nature of the reaction is rather determined by the necessities of the plant, and hence we meet with roots which are negatively geotropic, and grow out of instead of into the soil (e.g. the pneumatophores of palms, &c., KARSTEN, 1890) and positively geotropic shoots which burrow into the soil or at least grow in a downward direction (e.g., rhizomes of *Yucca* and *Cordyline*, and many flower stalks after pollination, &c.). Nor is the type of geotropism always constant for the same organ, for, as we shall see later, a normally positively geotropic organ may become negatively geotropic and assume some other relationships to the direction of the action of gravity, to which we have not as yet made any reference. On the whole it may be said that geotropism is a phenomenon of wide distribution in the plant world, for we meet with it not only in the highest plants but also in mosses, Algae and Fungi ; it appears both in multicellular structures and in unicellular organs (internodes of *Nitella*, rhizoids of *Chara*), and in unicellular (coenocytic) plants such as *Mucor* and *Phycomyces*. On the other hand, some plants, such as the mistletoe and many Algae, are not geotropic at all.

Our next task must be to examine more closely the precedent phenomena of geotropic curvature. That this movement depends on the unequal elongation of opposing sides of an organ is self-evident, but how this arises cannot be deduced from the actual curvature itself. Curvature, as we have seen in the preceding lectures, may arise from turgor or from growth. Geotropic curvatures, as a matter of fact, arise in both ways, but curvature due to changes in turgor occurs only in organs which we do not propose to discuss in this lecture. We will confine ourselves at present to a consideration of those which are due to *growth*, and endeavour to explain the principles of the subject by reference to a few examples. We will select for that purpose multicellular organs, for there are no exact researches available in unicellular organs. Geotropic curvatures have served far more frequently as a means of demonstrating theories than as the subject-matter of exact observation. Almost all that has been done in this latter relation is due to SACHS. There is no reason to suppose, however, that there are any differences in this respect between unicellular and multicellular organisms.

Let us begin with geotropic curvature in the *tap root*, which we will imagine, to begin with, is laid horizontally. Fig. 133 (SACHS, 1873) shows the different stages in geotropic curvature taken up by the root of *Vicia faba* grown in very loose soil at a temperature of 20° C. The growing region (p. 289) is divided into five equal parts, each 2 mm. long, from the growing apex backwards ; these may be indicated by the numerals I (from 0 to 1), II, III, IV, V respectively. A pointed paper index points to 0 (*A*). In *B* the same root is figured an hour later ; the root is still straight, but it has already elongated about 1·6 mm., as the change in the position of 0 shows. In *C* the root, after two hours' interval, is seen to have developed still further and to have curved considerably. If the

course of the root be watched through a transparent sheet of mica, on which circles have been scratched, it will be found that it takes the form of an arc of about 15 mm. radius. *D* shows the same root seven hours after the commencement of the experiment, and now it will be seen that the marks 1 and 2 have already moved past the index, and that the root as a whole has elongated more than 4 mm., the individual increments measured on the convex side being :—

Zone	V. (base)	IV.	III.	II.	I. (apex)	Total.
Increase in mm.	0·4	1·0	1·8	0·8	0·2	4·2

The curvature is further sharpened ; the radius of the convex arc, which was 15 mm. in C., is reduced to 10 mm. in *D*. The curve corresponds to the arc of a circle, in the formation of which all the growing parts take part up to mark 5, although apparently the zones II and III are more sharply bent than I, IV, and V. *E* illustrates the root after twenty-three hours, and the curvature now exhibits two changes ; in the first place, it is no longer represented by an arc of a circle, the curvature is much greater between marks 2 and 3 than in the region in front or behind ; in the second place the radius of the curve between 2 and 3 is still further reduced, viz. to about 8 mm. In stage *D* the apex of the root lies at an angle of about 45° with the horizontal, in *E* it is at right angles to the horizontal, and we can see that the cause, but not the only cause, of the downward direction taken by zones II and I is the bending and growth of zone III (between 2 and 3). In zone II curvature is still apparent, which decreases towards mark 1, while the bending in zone I is scarcely observable at all. From mark 3 to the apex the form of the root approaches a parabola whose apex lies somewhere near 3 (Sachs, 1873 b, p. 440).

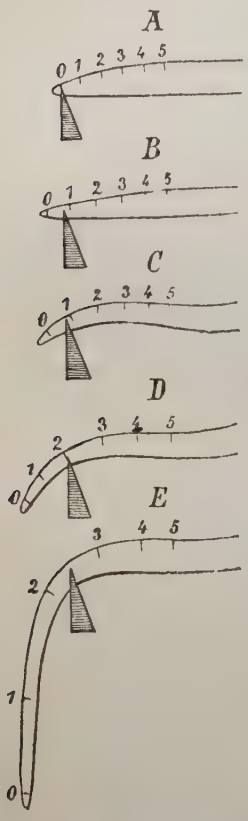


Fig. 133. Geotropic curvature in a root. After Sachs, from Detmer's Smaller Practical Physiology, 1903. (The index in *D* should be a little more to the right).

If we now inquire why it is that at stage *D* the growing region does not show equal curvature in all zones, we shall find that the reason lies in the different intensities of growth in the separate zones and also the different positions assumed by them. Zone IV, after seven hours, has increased markedly less than III, and V is already full grown. The capacity for bending has thus ceased in zone V, while in IV it is obviously much less than in III. Zones I and II, however, which in the end grow much more rapidly than III, in a very short time attain the vertical, that is, succeed in reaching a position where the geotropic stimulus can no longer affect them. It would appear, however, that another important condition must be taken into account in considering the localization of the chief region of curvature in zone III, a condition the value of which may be estimated with the greatest certainty in organs with longer growing regions than roots have. The straightening taking place in zone I must, as shown in the figure, be partly the result of elongation, for a curved organ must become gradually flatter, as simple geometrical considerations show us, if it grows equally both on the convex and concave sides.

From what we have now seen we may conclude that the bending of the root is limited to the growing zone, but our observations have taught us nothing

as to the more immediate course of growth. In this relation there are the following possibilities to be taken into account :—

1. Growth proceeds on *one* side with uniform rapidity.

(a) This side is the *concave* side, but an increased growth must then occur on the convex side.

(b) This side is the *convex* side, but the rate of growth must be reduced on the concave side.

2. Growth alters on *both* sides, decreasing on the concave and increasing on the convex.

In the second alternative the decrease of growth on one side may be as great as the increase on the other, and then the rate of growth in the axis of the root, which is equidistant from the convex and concave sides, does not alter at all ; but in the former possibility growth of the axis must always alter, showing an acceleration in (a) and a retardation in (b). In order to demonstrate this point clearly SACHS (1873 b) calculated the increments of growth on the convex and concave sides, and also in the axis of roots which had for some hours undergone geotropic curvature, and, for the sake of comparison, corresponding measurements were made on a root which was allowed to grow straight. The following is a summary of the results obtained :—

		Convex side.	Concave side.	Axis.	Straight root.
Increase in 4 zones	Root No. 1	10·8	6·1	8·4	10·5
	,, No. 2	8·7	5·3	7·0	8·5
Increase in 3 zones	,, No. 3	5·8	2·8	4·3	5·5
	,, No. 4	6·7	4·2	5·5	6·0
		32·0	18·4	25·2	30·5

This table shows that the curvature both on the *average* and in each *individual* case is due to a slight acceleration of growth on the convex side and a marked retardation on the concave side ; axial growth is more restricted than in the root allowed to grow normally. [According to LUXBURG'S (1905) measurements SACHS'S results are not to be depended on. This author holds that decrease of growth does not take place in the middle line.]

Negative geotropic curvature in a *stem* is illustrated at Fig. 134 (SACHS, 1888). The region in this example which is capable of growth is about 50 cm. in length. It has been divided into five zones by indian-ink lines, the four lower (5–2) being each 100 mm., the uppermost (1) only 50 mm. long. The stem was laid horizontally at noon (*a*). After $3\frac{1}{4}$ hours (*b*) curvature had taken place in all the zones ; zone No. 1 had shown the greatest curvature (radius = 16 cm.), the least curved was zone No. 5. After $5\frac{1}{2}$ hours (*c*) the greatest curvature was observable in zones Nos. 3 and 4, while zone No. 1, which had already bent beyond the vertical, had begun to straighten itself. After twenty-two hours (*d*) zones 1–3 had become erect and the chief curvature (with 7 cm. radius) lay between the bottom of 4 and the apex of 5. There are two phenomena worthy of note in this experiment. In the first place, the removal of the region of most vigorous curvature to the still growing base from the zone of maximum growth near the apex, where it first appears, and, in the second place, the supra-curvature of the apical region, occasioned not only by the after-effect of the geotropic stimulus but also by the basal progression of the bending. This supra-curvature is in some cases much more apparent than in the case of *Cephalaria*, as may be seen from a glance at Fig. 135. The supra-curvature is, however, very soon neutralized, for a new geotropic stimulus begins to operate in the opposite way, and for other reasons which we have already hinted at (p. 432), but of which we shall have to speak later on.

The final result is invariably that a definite basal curving takes place

at the boundary between the completely grown and still growing regions, and that the entire apical region becomes perfectly perpendicular.

In order that we may clearly appreciate the distribution of growth in the shoot we will study more in detail SACHS's numerical results from experimental research on the stem of *Cephalaria*, as figured at Fig. 134. The letter U indicates the *increase in length* on the *under* side, O that on the upper side, *both in mm.*; and it must be noted that the uppermost zone at the commencement is only half as long as the others; R represents the radius of the arc of the curvature *in cm.*

	Zone 5.			Zone 4.			Zone 3.			Zone 2.			Zone 1.		
	U.	O.	R.	U.	O.	R.	U.	O.	R.	U.	O.	R.	U.	O.	R.
Stage b	1·2	0·0	47	1·5	0·0	40	4·3	0·4	30	4·1	1·6	21	3·0	0·0	16
Stage c	1·5	0·0	46	4·5	0·0	18	6·0	1·0	15	5·0	1·3	18	4·4	1·3	17
Stage d	10·0	−1·0	7	9·5	3·0	7	15·0	15·0	∞	15·0	13·6	∞	5·0	4·0	∞

Since we have no data available as to growth in an uncurved control

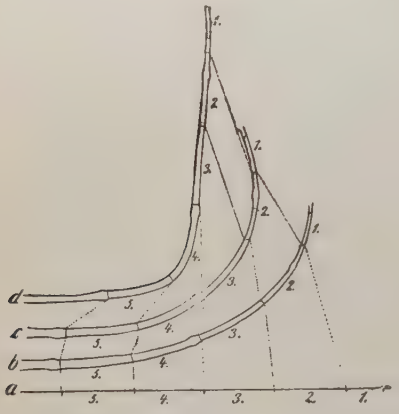


Fig. 134. *Cephalaria procera.* *a*, stem laid horizontally at noon, showing divisions; zones *5*, *4*, *3*, and *2* each 10 cm. long, zone *1*, 5 cm. long; *b*, the same 3¼ hours later; *c*, 2¼ hours later than *b*; *d*, 16 hours later than *c*. After SACHS (1888). About one-tenth nat. size.

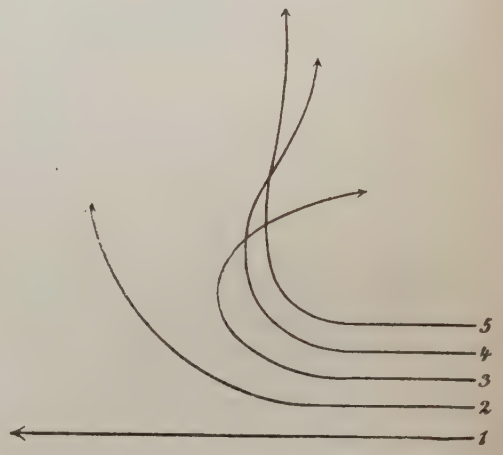


Fig. 135. Geotropic curvature in *Allium atropurpureum* in various stages (*1–5*). After SACHS (1882, p. 839).

experiment it is impossible to say here, as in the case of the root, whether an elongation takes place *over all* (measured on an axial line), and we can, therefore, only affirm that, during curvature, growth on the *concave* side frequently remains stationary, or that it shows a distinct retardation. We must not attempt to generalize on these results, since growth takes place on the concave side, not merely in the example we are considering, but in other organs also, and the principal fact is that there is a differential growth on both sides. As a second illustration we may take the measurements made by NOLL (1888). This author established the fact that in *Hippuris*, a geotropic curvature occurred when the increase on the *under* side was (in twelve hours) 5 mm. and on the upper side 0·25 mm. In the same time the axis increased about 2·6 mm. Comparing these figures with growth in an erect shoot of *Hippuris* we find growth in the latter to amount only to 1·0 mm. Here, therefore, we have to deal with an acceleration of growth and not, as in the root, with a retardation. [LUXBURG (1905) was *unable* to confirm this growth acceleration in *Hippuris*; on the contrary, this author has shown that both here and in other plants during the curving a retardation of growth occurs.]

Differential growth has been established not only between the upper and under sides of an uninjured and complete organ but also in isolated portions; thus in stems, at all events, curvatures have been observed in parts cut out from a shoot, while roots, after wounding, remain for a longer time insensitive to the influence of gravity. It is by no means remarkable that separated segments of stems should still show geotropic curvature, but the behaviour of plant organs when split longitudinally is worthy of note. If a *stem* be split longitudinally a curving outwards of each half must occur in consequence of differences in tissue tension, but if one of these halves be arranged so that its epidermis is uppermost, and the other half be in the reverse position (the cut surface of the medulla lying horizontally), geotropism influences each half differently, and induces in them differential growth between the upper and under tissues; in the section which lies epidermis upwards growth in the medulla is accelerated while the epidermis shortens, in the other half the epidermis increases in length and the medulla grows less vigorously than in the other half. Tissue tensions, however, in this case, to a certain extent, obscure purely geotropic curvature. If similar researches are made with nodes of grasses, i. e. with the swollen basal regions of the leaf sheaths where tissue tensions of this type are absent, geotropic curvature may be determined both in the upper and under longitudinal halves; it makes no difference which side is uppermost. De Vries (1880) has shown that geotropic curvature occurs in each longitudinal area even if the shoot be divided in four.

Grass nodes are of interest from another point of view. In the organs hitherto spoken of the geotropic curvature depends on longitudinal growth; where longitudinal growth ceases there curvature is also absent. At all events, this is the conclusion to which all investigators have come who have examined the question, with the exception of Kohl (1894), who holds an opposite view. The nodes of Gramineae are able, however, to develop geotropic curvature in the full-grown condition, for they are capable of renewing growth each time they are removed from the position of geotropic rest. In this curvature the under side undergoes great elongation; in a very short time it becomes double or even as much as five times as long as it was, while the upper side is forcibly compressed so much as to throw it into *folds*. A few numerical details (Sachs, 1872, 206) will make this clear.

Cinquantino Maize. Length of nodes in mm.	Upper.	Under.	Upper.	Under.	Upper.	Under.
Before bending	4·3	4·1	4·0	5·0	5·0	5·0
After bending	2·5	9·0	3·0	11·0	4·5	12·5
Difference	−1·8	+4·9	−1·0	+6·0	−0·5	+7·5

More recently, from many points of view, it has been shown that not only grass nodes and related structures, but many other full-grown organs, may develop curvature when subjected to geotropic stimulus. Branches also which are secondarily thickened may exhibit geotropic curvature, which cannot be induced in plants, e. g. palms, which have no power of secondary growth. It must be assumed that the power of curving rests in the power the cambium has of producing elements of different lengths on either side. Detailed investigations on these points are, however, not available. (Compare Meischke, 1899; Jost, 1901; Baranetzky, 1901.)

In every case which has been accurately studied the immediate cause of the curvature is a difference in longitudinal growth of opposite sides. As is generally the case a stretching of the cell-membranes due to turgor precedes surface growth, and this is gradually rendered permanent by growth. If the organ be plasmolysed at the commencement of geotropic curvature it grows at first in a straight line, later on, however, the curvature is permanent.

Turgor extension is unequal on the two antagonistic sides. The difference would appear to depend on the fact that the *osmotic pressure* increases on the convex and decreases on the concave side, but that is by no means the case; on the contrary, the pressure on the concave side appears to remain constant, while that on the convex side is reduced. Since the rate of growth of the cell-wall does not depend directly on the amount of osmotic pressure, there is nothing very astonishing in this. Unequal turgor *extension* of the two sides must depend on an alteration in the elasticity of the cell-walls. We have already seen that there are no reliable data available as to the causes for such alterations in elasticity any more than there are on the general question of the mechanics of growth in the cell-wall. On that account it is needless for us to enter into such controversial points, although geotropic curvatures have frequently been brought forward in support of different views as to the mechanics of growth in the cell-wall. Unfortunately, measurements made of curving organs often do not at once determine whether the concave or the convex side, or both, are actively concerned in the bending. In many cases, such as those where the concave side is directly *shortened*, there can be no doubt that it behaves *passively*, and that the curving is the result of vigorous stretching of the convex side aided by rigidity in the axial region. If, as in grasses, the concave side be thrown into folds, the passivity of that side makes itself apparent at once. It does not always behave in this manner, however. Certain experiments of SACHS (1873 a), where the several tissues were removed during the bending, tend to show that the axis (medulla in the case of the stem) is not directly concerned in the process, but this is true only of the uninjured plants and not of the longitudinally spilt internodes mentioned on p. 435.

Assuming then that curvature in general depends on unequal growth on opposite sides, that, in positive geotropism, growth is retarded on the side of the organ facing the soil, while it is accelerated on the upper side, and that in negative geotropism the distribution of growth is reversed, we have next to ask how it is that gravity influences growth, and especially how it is able to influence different organs in different ways. As a matter of fact, the question has been in a sense already answered by our describing geotropism as a stimulus reaction; the significance of this terminology being that gravity is to be regarded merely as a releasing force and not one which acts in a purely mechanical manner, and this conception of the phenomena must obviously be looked upon as the correct one, when we remember that gravity induces diametrically opposite reactions in positively and negatively geotropic organs. The history of the science, however, shows (as to the history of geotropic investigations compare SCHOBER, 1899) that this conception was only arrived at as the result of considerable labour and was by no means self-evident from the very first. As a matter of fact, even as late as the seventh decade of the last century, an investigator of the rank of HOFMEISTER (1863) attempted to show that gravity acted in a purely mechanical way. This author believed that the softness of the root accounted for its capacity for bending, which in turn was induced by the weight of the apex. Into his explanation of the negative geotropism of the stem we need not enter, for it has only an historical significance. The theory based on the plasticity of the root apex is also of interest only from an historical point of view, although it is full of lessons for us even nowadays, as showing how thinking men under the dominance of a preconceived notion may go blindly in opposition to facts; it reminds us also that the *fact* is the chief thing, the *theory* only, as it ought to be, the ever-changing expression of the aggregate of experience. Had HOFMEISTER not been imbued with a preconceived idea he must have seen that the apex of the root really more closely resembles a piece of glass than a stick of hot sealing-wax, JOHNSON (1828) had long previously shown that the weight of the root apex may

be supported by a force equal in amount and yet that the geotropic curvature was still maintained, and in 1829 PINOT found that the root apex was able, when bending downwards, to force its way into mercury, and could overcome a very considerable opposing pressure. HOFMEISTER was, however, unacquainted with these older observations, and FRANK (1868) was the first to replace the view of passive sinking of the root apex held by HOFMEISTER by the correct interpretation, when he said that in geotropism we were dealing with a special evolution of energy which was released in the interior of the organ by gravity. Subsequently the effect of gravity as a releasing force was fully discussed by PFEFFER (1875, 1893 a) and by SACHS. It ought also to be mentioned that, in 1824, DUTROCHET spoke of gravity as a releasing agent, although, later, he enunciated another view.

As the downward curving of the root may be accompanied by a recognizable expenditure of energy, so also negative geotropism is the result of a similar expenditure, because a weight has to be *lifted*, and because the force acts on a very much larger lever in the shoot than in the root. PFEFFER (1893 b) has recently studied the work done in the nodes of grasses subjected to geotropic curvature, and MEISCHKE (1899) has examined other plants with a similar end in view. It has been proved that the energy required for carrying out the work accomplished reaches values such as we might expect to obtain on the principle (p. 422) that geotropism is a growth phenomenon. It has been shown more particularly that the energy expended in making a stem of a grass stand erect is about that required for the purpose, but that, as a general rule, a large surplus of energy is developed in other geotropic curvatures which permits of the straightening of the shoot after pronounced over-curving of the apex. Although we cannot go into details here it will be sufficient for us to know that the work done during the curving bears no ratio to the energy provided by gravity. The energy required to produce the movement is supplied by the growing parts of the plant itself, gravity acts merely as a releasing force.

If gravity acted only through the weight of the moved organs the nature and amount of the resulting curvature might be determined according to mechanical principles, but if it acts as a stimulus we must first of all determine experimentally how far the curvature depends on the duration, intensity, and direction of the force. Even in rapidly reacting organs there is always an interval of about one to one and a half hours, before the horizontally placed organ shows a noticeable curvature, and this latent period may in other cases be extended to several hours. It is by no means essential that the plant should be stimulated continuously until the reaction begins—it is quite sufficient if the stimulus be applied for a shorter period. As CZAPEK (1898) has shown, the sporangiophores of *Phycomyces*, the hypocotyl of *Beta*, the first sheathing leaf in seedlings of *Avena sativa* and *Phalaris canariensis*, after exposure to the geotropic stimulus for only fifteen minutes, exhibit later a curving, even if meanwhile they be placed in a vertical position, or, better still, rotated on a klinostat. An *after-effect* following on a geotropic stimulus makes itself apparent not only if the organ during the curving is gradually placed in its normal rest position but also if the stimulus be removed long before any visible reaction occurs. The minimum time during which the geotropic stimulus must be applied in order that a bending may take place as an after-effect, we term the *latent period*. It has in no case been found to be less than fifteen minutes, and amounts to twenty minutes in the radicles of *Zea*, *Pisum*, *Lupinus*, and *Cucurbita*, to fifty minutes in the epicotyl of *Phaseolus*, and to several hours in other structures. [According to FITTING'S (1905) researches CZAPEK'S numbers are not to be relied on; the latent period may often amount to only 6–7 minutes, and, according to MOISESCU (1905), it may be even less than that. There is no doubt that the latter author's employment of the microscope for the determination of the commence-

ment of curving suggests further lines of research, but his statements are open to criticism.] The period of duration of the stimulus affects very markedly the beginning of the geotropic curving, for while roots of *Lupinus*, kept in the horizontal position for thirty-five, forty, fifty, sixty minutes, bend rapidly one after the other on the klinostat, so that after ninety minutes the reaction is visible in every one of them, the curving does not begin for two or three hours when the roots are exposed for only twenty minutes. We may suppose that a stimulus applied for a still shorter period, e. g. less than the latent period, must still have some slight effect on the plant, although it does not induce a visible curving. Indeed, there are observations recorded which confirm this supposition, i. e. experiments on intermittent stimuli. If the root of *Linum* be placed horizontally for two minutes and vertically for six minutes alternately, geotropic curvature takes place after a certain time, although each separate stimulus is far shorter in duration than the latent period, and hence is unable alone to induce a curving. If each individual stimulus made no permanent impression on the plant it would not be possible for a summation of these stimuli to result in a geotropic reaction. We must assume that every stimulus, however short it be in the period of application, produces some internal change, which we may describe as an *excitation*. This excitation lasts longer than the stimulus, and hence a summation of excitations is possible, and when this summation reaches a certain amount then the liminal intensity is exceeded and curvature begins. Detailed research is still required before we can say how small the stimulus periods may be when these follow each other at regular intervals and also how great the intervals may be between the individual stimuli, for it stands to reason that there must be limits to both. The matter is of importance, for the whole theory of the klinostat rests on the results of such experiments. We are as yet quite ignorant, for instance, whether plants are really geotropically stimulated at all when placed on a klinostat or whether the individual stimuli neutralize each other. CZAPEK till recently (comp. 1902, 468) held the former view. We may imagine with him the uniform rotation of the klinostat replaced by four successive jerks, so that the plant for a certain time is allowed to rest in each of the four chief positions, viz. above, right, below, left. The plant, according to CZAPEK, must remain in each of these positions for a briefer time than the length of its latent period; thus if the latent period be twenty minutes one complete rotation may be effected in sixty minutes, so that the plant remains for fifteen minutes in each of the four positions, i. e. less than the latent period and hence is not stimulated. According to NOLL (1900), however, we have in this case to deal with intermittent stimuli, for at forty-five minutes intervals, a definite face is brought under the influence of gravity for fifteen minutes, but bending cannot take place because each stimulus is again neutralized by the corresponding stimulus applied when the plant is in the opposite position. In many organs it is quite immaterial, so far as the result is concerned, whether on the klinostat the reaction only or the stimulus as well ceases; the nodes of grasses, however, behave quite differently in either case, they must be able to discriminate between the two possibilities. If laid horizontally they not only bend but also exhibit renewed growth. If they be placed in a klinostat they begin to grow (ELFVING, 1884), but their growth is uniform on all sides. This behaviour of the grass nodes appears to prove (compare PFEFFER, Phys. II, 126, and NOLL, 1902, p. 413) that the movement of the klinostat inhibits the geotropic *curvature* but not the geotropic *stimulus*. If this conclusion be correct, then undoubtedly *an alteration in the rate of growth on the klinostat* may be universally demonstrated where an organ laid horizontally grows axially more slowly or more rapidly than when placed vertically; it (e. g. *Hippuris*) must also grow more rapidly on the klinostat than under normal conditions. Only such organs as exhibit a retardation on the concave side equal to the acceleration on the convex side may go on growing in an unaltered form on the klinostat.

Experiments on this subject are as yet scanty, so that we need not attempt to arrive at any definite decision between the two theories. [Meanwhile FITTING'S (1905) careful investigations have made us more thoroughly acquainted with these geotropic phenomena. This author has studied in detail the results of intermittent stimulation, and was able to show that the duration of a single stimulus may be shortened *at will*, and that by summation of these stimuli a movement is finally induced. FITTING has also studied the relation between the period of duration of the stimulus and of the interval of non-stimulus in the case of intermittent stimuli, and has found that geotropic curvature invariably takes place if the intervals are ten times as long as the periods during which the stimulus is applied. He has also shown quite clearly that only the bending and not the perception of the stimulus is impossible on the klinostat.]

In addition to the *duration* of the geotropic stimulus we must also take into consideration its *intensity*. The variations in the amount of the stimulus, however, as observed in different regions of the earth, are so minute that it is quite impossible to deal with them experimentally, even if they were more accessible to the observer than they really are. KNIGHT'S discovery relieves us from all difficulty in this respect, for we may increase the *centrifugal force* to any extent we please, and we may thus study the dependence of the latent period on the amount of this force. If we vary the centrifugal force so that on the one hand it amounts to thirty-eight times the value of g. (gravity) and on the other hand reduce it down to 0·0005 g. the reaction takes place (in the root of *Vicia faba*, CZAPEK, 1895) in the following times :—

¾ h.	1 h.	1½ h.	1¾ h.	2½ h.	3 h.	4 h.	5 h.	6 h.	8 h.
38–35 g.	28–10	7–4·3	3·5–0·9	0·6	0·5–0·4	0·2–·02	0·003	0·001	0·0005

From these experiments it follows first of all that the plant responds in the same way but more slowly to a pulling force a thousand times less than g. As in all movements manifested in response to a stimulus so in the case of geotropism the stimulus must reach a certain amount, the so-called 'liminal value', before a reaction follows. The reaction follows all the more rapidly the more vigorous the releasing force is, and we may, therefore, conclude that the effect of the stimulus or the excitation in the plant is so much the greater the more vigorous the centrifugal force is. No investigations have as yet been made on the effect of still greater intensities of centrifugal force. It must *not* be assumed that the excitation increases *pari passu* with the increase in the centrifugal force, because this force will in the long run have an injurious effect on the plant, or at least retards growth (ANDREWS, 1902). It may also be possible to determine experimentally an apex on the stimulus curve (region of greatest excitation) and an upper limit of stimulus in addition to the already known liminal intensity.

We may alter not only the *duration* and *intensity* but also the *incidence* of the geotropic stimulus. If a shoot be so placed that it grows in a straight line *upwards*, that is to say, parallel, but in the opposite direction, to that of the geotropic stimulus, there is no reaction at all, or, to be more accurate, there is no geotropic curvature. If, however, the shoot be placed at an inclination to the vertical so that the line of direction of gravity makes an angle with the axis, a curvature takes place, owing to the fact that on the under side growth is accelerated and on the upper side retarded. The influence of gravity will have all the greater (but purely mechanical) effect the more nearly the stem approaches the horizontal. In that position gravity should have its maximum effect, and if we go on turning it over, that effect will again be diminished, until finally, in the inverted position, it will have reached zero. Recent researches do not, however, confirm this view. CZAPEK (1895), employing various methods of producing the excitation, found that the maximum effect was produced when the angle 135° downwards was reached. Roots behave exactly in the reverse way, responding most when placed

at an angle of 135° with the vertical and pointing upwards, i. e. 45° above the horizontal. [Several authorities, FITTING (1905) especially, have shown that the optimum stimulus is given in the horizontal position.] In both types of organ, however, it may be determined that in addition to the normal position the inverse position is also a position of rest; certainly roots bend downwards in a short time when inverted and shoots upwards.

These reactions are, however, consequent on small curvatures induced by internal factors, by which a deviation from the position of rest is brought about. If a plant turned upside down be mechanically prevented from performing any autonomous curvature a geotropic curvature never takes place as an after-effect on the klinostat. There is always one noticeable difference between the two positions of rest, the normal position is stable but the inverted position is labile. Any organ which is inclined somewhat from the inverse position does not bend back again into that position but attempts to assume the normal direction. The only point we have to deal with here is the transference of the stable into the labile position, and vice versa; we have only to alter the end by

Fig. 136. Two shoots of *Physostegia*, fixed horizontally in wet sand and in a moist atmosphere, one (the right) by its base, the other (the left) by the apex. Both show geotropic curvature. (Slightly reduced).

which the plant is fixed (FRANK, 1868; NOLL, 1892). If we employ isolated branches and fix them horizontally by their *apices* the reaction which takes place is the same as that seen when the branch is fixed in the normal position, that is to say, the side facing the ground exhibits growth acceleration, but the further results are quite distinct, for *the base bends upwards and reaches the stable rest position in the inverted lie* (Fig. 136).

Any attempt to discover why a geotropic curvature follows when the plant is in certain positions, while other positions may be described as positions of rest, at once suggests the question, what is the initial effect of gravity on the plant? Researches which have been carried out during recent years show more and more clearly that between the application of the stimulus and the movement a whole series of processes takes place, whose existence is rendered especially prominent if the application of the stimulus and the performance of the movement affect distinct and widely separated parts of the plant. Under such circumstances at least three different operations may be distinguished; (1) the appreciation or perception of the stimulus by the receptive or sensitive organ; (2) the reaction of

the motile organ; and (3) obviously the transference of the former to the latter. The credit of having been the first to clearly distinguish between stimulus and response belongs without doubt to CHARLES DARWIN (1881), although the evidence he put forward in support of the fact has not stood the test of later criticism.

Such localization and separation of perception from response have now been demonstrated with certainty in the case of heliotropic stimuli (Lecture XXXVI); whether the same is true of geotropism also is not quite so well established. In spite of CZAPEK's (1895, 1900) ingenious experiments, which we cannot pause to describe here, it is very doubtful, for many reasons, whether the seat of geotropic perception lies really in the root apex or only in the root-cap (NĚMEC, 1900, 1901); similarly, there is considerable room for suspecting the truth of the assertion, so often made, that in the case of the seedlings of certain grasses (Paniceae) it is only the extreme apex of the cotyledon that is sensitive to the geotropic stimulus. [Even yet this question has not been decisively answered; new methods have been invented by PICCARD to determine this point (1904), but his results cannot be considered as above criticism. Compare also RICHTER (1902), DARWIN (1902), and MASSART (1902).]

Although a considerable separation between the region of perception and of movement has not as yet been demonstrated in the case of the stimulus of gravity there are certain recorded observations of CZAPEK (1898) available which go to show that there are at least two different series of processes: those concerned with the perception of the stimulus and those concerned with the reaction. As in the case of all movements in response to stimulus, geotropism depends on certain general or formal conditions; there must be a certain temperature, a certain nutritive supply, water and oxygen in sufficient quantity, &c., &c., before any geotropic response can take place. The conditions which have to be fulfilled before the stimulus can be appreciated are *not the same* as those which govern the occurrence of the reaction, for the stimulus may be perceived under circumstances where no growth or geotropic response may take place. Thus at 2° C. a geotropic response may be *induced* after a sufficiently long exposure to the stimulus of gravity, but the movement is *carried out* only when the plant is exposed to a higher temperature. Again, the stimulus of gravity may be appreciated in an atmosphere free from oxygen, but that gas is essential for the carrying out of the movement. In this way, or by methods of a similar character, we may convince ourselves of the existence of two separate phenomena, phenomena of perception and phenomena of reaction.

How comes it to have sensitivity of this kind? What is it that the plant perceives when gravity affects it? KNIGHT's experiments leave no doubt in our minds that gravity influences the plant only through mass acceleration, which it exercises on all bodies, i.e. by weight. But it is also certain, as we have seen, that the weight of the overhanging part of the plant above the zone of curvature has nothing to do with it, since we can neutralize that without stopping the geotropic movement. What we have to deal with is an effect of weight operating in the interior of the plant, even in each individual cell. Since, however, it is not infrequently the case that most of the protoplasm exhibits streaming movements it follows that it is only the quiescent ectoplasm, as NOLL has pointed out (1888, 532), that can be sensitive to the stimulus of gravity; it must be able to distinguish between varying pressure on different sides of the cell. Let us now assume the whole cell contents, vacuoles, and streaming protoplasm to be the cause of the weight, in the vertical position of the cell a lateral pressure must be exerted on the ectoplasm, but any two opposite regions must be subjected to similar pressures. Let us now incline the cell somewhat out of the perpendicular, then a pressure must be exerted at once on a certain part of the under side somewhat greater than that on the corresponding part of the upper side. But when we remember that a considerable

pressure on the cell-wall always results from the osmotic activity of the cell-contents, we can scarcely assume that the limited *alteration* in pressure resulting from the inclining of the cells can be perceived by the plant. If, like NOLL (1902), we make the very modest assumption that the turgor pressure amounts only to three atmospheres, there rests on the ectoplasm in every position a water column of 30 m.; if we assume the diameter of the cell to be 0·1 mm., the under side of the organ in the *horizontal* position must bear an excess of 0·1 mm. of water over the upper side; the plant must be able to appreciate the difference between a pressure of 30,000 and 30,000·1 mm., and if the inclination of the cells be less or the turgor be higher it must be able to appreciate even less marked differences of pressure.

Apart altogether from this conception there are two hypotheses which have recently been advanced to account for the power plants have of appreciating the stimulus of gravity. NOLL (1900) imagined that a sensitive apparatus was formed in the ectoplasm, analogous to the statocysts which occur in crayfish, adapted to the appreciation of the direction of gravity but beyond the limits of vision. These must consist of approximately spherical vesicles composed of sensitive plasma filled with sap, and containing a small but relatively heavy body in the fluid. This body would correspond to the statolith of the crayfish, and we may also term it a 'statolith', and it must, according to the position of the plant organ in space, exert a pressure on some definite part of the sensitive plasma, and so induce a 'perception' in the plant. In order to explain this theory more in detail let us select for study a sporangiophore of *Phycomyces*, laid horizontally. The pressure on the outer side of the sensitive plasma would operate in the statocysts of the under side, and a growth acceleration would be induced as a response to this; on the upper side, however, the insides of the statocysts would be affected by the statoliths and a retardation of growth would result. If the statolith affects the intermediate limit between the regions which induce either an acceleration or retardation of growth, i. e. between the outer and inner hemisphere, then there is no sensation or at least no reaction. In the horizontal position this would be true of the side walls, but in the vertical position the whole of the statocysts come to lie in neutral regions above or below in the cyst; in the former case only the statocysts of the sides are unaffected, in the latter all are unaffected. The application of this hypothesis to a positively geotropic cell presents no difficulty and so it need not be discussed here, but certain criticisms may be briefly advanced against this conception of NOLL's.

We are right in opposing the assumption of special relationships which lie beyond the limits of vision until the hypothesis in question presents us with an explanation on broader grounds so comprehensive that we can no longer do without it. As an example we may refer to the atomic theory. But we cannot compare NOLL's hypothesis in any way with that theory, because the former attempts merely to explain the phenomena of geotropism and has no further and wider application. Apart from that, one difficulty presents itself on passing from a consideration of the uni- to that of the multi-cellular organism. Since this latter organism exhibits geotropic curvatures not merely in the uninjured condition but also when cleft longitudinally, we must assume that *every individual cell* in it is supplied with statocysts. In each individual cell, at all events in the cells of a median vertical lamella of the horizontally placed stem, growth acceleration on the under side and growth retardation on the upper side should be opposed to each other; in reality, the cells near the under edge show *accelerated growth* on *both* sides, those near the upper edge show *reduced growth* on *both* sides, and the median zone remains unaffected. Hence we cannot assume the close relationship between perception and reaction that NOLL does. Response is regulated by the co-operative action of all cells, correlation plays a part which NOLL's hypothesis does not explain. The hypothesis, too, has a certain one-sidedness in the close

connexion between perception and response. Further, we do not know enough as to the geotropic *response*. NOLL appears to assume that the cells of the concave side are just as active in the reaction as are those of the convex side, but the retardation of growth in them can be also passive and due merely to the accelerated growth of the convex side (p. 435). NOLL's hypothesis brings up quite special difficulties, however (compare PFEFFER, 1893 a), if the perception really takes place elsewhere than does the movement, for according to certain observations of CZAPEK (1898), it is quite certain that the perception of the under region of a horizontally laid root apex cannot be different from that of the upper side, and that it is *only* transmitted to the under side of this motile zone. On the contrary we must assume *equal* perception in all the cells of the apex, and yet the cells of the motile region react quite differently.

Many of the criticisms we have advanced against NOLL's hypothesis are applicable also to HABERLANDT'S (1900) and NĚMEC'S (1900) views, simultaneously and independently promulgated. These authors also employ a statocyst hypothesis. The sensitive plasma is the ectoplasm of the entire cell, the statoliths are relatively heavy bodies, such as crystals and starch grains. Starch grains, which respond quickly to the influence of gravity and press against a different region of the ectoplasm when the plant is in the upright than when it is in an inclined or horizontal position, are found in many plants, regularly in the starch sheath of the stem, and in the root in a central group of cells of the rootcap. Fig. 137 shows them in the apex of a cotyledon of a grass. The cells which contain motile starch grains of this kind are conceived by the authors above mentioned to be the sense organs for the geotropic stimulus. This hypothesis has this great advantage that it can be examined into with the aid of a microscope. It has called forth a whole series of most interesting observations (HABERLANDT, 1903; DARWIN, 1903), into the discussion of which we have unfortunately no time to enter. But difficulties of various kinds meet us here also (JOST, 1902). We will confine ourselves to showing that evidence is entirely wanting tending to prove that *only* cells with movable starch grains are able to appreciate the stimulus of gravity. It will be sufficient to draw attention to the fact that there are plenty of plants which exhibit the geotropic reaction, but which contain *no* starch, just as, conversely, there are cells with movable starch grains which give no geotropic response, although capable of growth. [The starch-statolith theory of geotropism has given rise to a flood of publications, and, although it has certainly found more supporters than opponents, we cannot as yet accept it as proved. Since, in the course of the controversy, HABERLANDT has admitted that a perception of gravity may occur without any change of position of the starch grains, he has placed the theory in an unassailable position, but at the same time rendered it impossible of proof. Further details will be found in the literature, of which we quote only the most recent and most important (HABERLANDT, 1905; NĚMEC, 1905; DARWIN, 1904; NOLL, 1905; FITTING, 1905).]

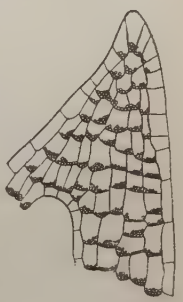


Fig. 137. Apex of the cotyledon of *Panicum miliaceum*. After NĚMEC (1901).

Although a study of the newer investigations, which have aimed at analysing the process of stimulation in geotropism, has led us to no conclusive result, we must yet admit that our knowledge has been essentially widened on the subject, for we have had convincing evidence that the whole process is a most complicated one and consists of many inter-related phases. In the first place we have the purely mechanical phase: gravity acts through weight affecting an unknown part of the cell—probably the sensitive plasma. It is not at all improbable that *pressure* on the plasma induces sensation through the weight

exerted thereby, although *accessory* changes may first of all lead to this result. In any case the first influence of the stimulus of gravity must be a purely physical or purely chemical alteration in the protoplasm, which we may term *perception*. Following on this perception comes an *excitation* in the protoplasm, which is undoubtedly distinct from the mere perception of the stimulus, since it may be cumulative, as we shall see in Lecture XXXVI. We know, further, that it is not coincident with the preliminaries of bending, that it requires different formal conditions, and may take place in a different part of the plant. In the latter case the excitation must be *transmitted*. If we consider the transmission of the excitation as the third phase then we must rank the final reaction as the fourth. We are quite ignorant whether no perception or no excitation occurs in the cases where no reaction is manifested, as, for example, when the organs are in the perpendicular position ; still we must believe that some kind of *response* takes place in the resting position also, but it must affect all parts *uniformly* and hence bending does not occur. Since stem and root grow just as quickly on a klinostat as in the erect position it *cannot* be concluded that the reaction is absent under normal conditions, especially since we do not know for certain whether stimulation occurs or not to plants on a klinostat. Since, however, *Phycomyces*, *Chara* and branches of weeping trees grow more slowly in the inverted position than in the normal, it follows that gravity acts as a stimulus in these cases.

It must also be noted that attempts have been recently made to solve the problems of geotropism by chemical and histological methods. Němec (1901) has shown that special rearrangements occur in the protoplasm of cells which respond to the geotropic stimulus. Doubtless, we are here dealing not with a primary effect of gravity but with complex stimulus-phenomena which bear some as yet unknown relation to the obvious curvatures. These phenomena are of great interest, however, because it was formerly suggested that gravity in the first instance induced movements in the protoplasm.

Czapek (1898, 1903) has discovered that certain *chemical* changes take place in parts which have been geotropically stimulated, and he has succeeded in proving that tyrosin is oxidized into homogentisinic acid. This oxidation *always* takes place in the plant, but homogentisinic acid is formed more abundantly after the geotropic stimulus has been applied, and apparently this increase is dependent in some way on geotropism, although what the connexion is is by no means clear. It cannot have anything to do with the *perception* of the stimulus, since it takes place in heliotropic curvatures also, which presuppose another sort of perception (Lecture XXXVI). If connected with the *reaction* it cannot occur first in the root apex and must also be distributed *unequally* in the zone of movement. The subject offers a wide field for experimental research. [Compare Czapek, 1905.]

Bibliography to Lecture XXXIV.

Andrews. 1902. Jahrb. f. wiss. Bot. 38, 1.
Baranetzky. 1901. Flora, 89, 138.
Czapek. 1895. Jahrb. f. wiss. Bot. 27, 269.
Czapek. 1898. Ibid. 32, 175.
Czapek. 1900. Ibid. 35, 313.
Czapek. 1902. Ber. d. bot. Gesell. 20, 464.
Czapek. 1903. Ibid. 21, 243.
[Czapek. 1905. Annals of Botany, 19, 75.]
Darwin, Ch. 1881. Bewegungsvermögen d. Pflanze. German ed. by Carus, Stuttgart.
Darwin, Fr. 1903. Proc. Roy. Soc. 71, 362.
[Darwin, Fr. 1902. Jour. Linn. Soc. Bot. 34, 266.]
[Darwin, Fr. 1904. Rep. Brit. Assoc. Cambridge.]

DUTROCHET. 1824. Recherches sur la structure intime etc.
ELFVING. 1884. Öfversigt finska Wetensk. förhandlingar.
[FITTING. 1905. Jahrb. f. wiss. Bot. 41, 221 and 331.]
FRANK. 1868. Beitr. z. Pflanzenphysiologie. Leipzig.
HABERLANDT. 1900. Ber. d. bot. Gesell. 18, 261.
HABERLANDT. 1902. Ibid. 20, 189.
HABERLANDT. 1903. Jahrb. f. wiss. Bot. 38, 447.
[HABERLANDT. 1905. Ibid. 42, 321.]
HOFMEISTER. 1863. Jahrb. f. wiss. Bot. 3, 77.
JOHNSON. 1828. Comp. Linnaea, 1830, 5, 145, Abstract of literature.
JOST. 1901. Bot. Ztg. 59, 1.
JOST. 1902. Biolog. Centrbl. 22, 161.
KARSTEN. 1890. Ber. d. bot. Gesell. 8 (55).
KNIGHT. 1806. Ostwald's Klassiker, Nr. 62. Leipzig, 1895.
KOHL. 1894. Die Mechanik der Reizkrümmungen. Marburg.
[LUXBURG. 1905. Jahrb. f. wiss. Bot. 41, 399.]
[MASSART. 1902. Mém. couron. in 8vo, Acad. Bruxelles.]
MEISCHKE. 1899. Jahrb. f. wiss. Bot. 33, 337 (363, note 1).
MOISESCU. 1905. Ber. d. bot. Gesell. 23, 364.
NĚMEC. 1900. Ber. d. bot. Gesell. 18, 241.
NĚMEC. 1901. Jahrb. f. wiss. Bot. 36, 80.
[NĚMEC. 1905. Studien über Regeneration, p. 334. Berlin.]
NOLL. 1888. Arbeit. bot. Instit. Würzburg, 3, 496.
NOLL. 1892. Ueber heterogene Induktion. Leipzig.
NOLL. 1896. Das Sinnesleben d. Pflanzen. Ber. Senkenberg. Gesell. Frankfurt.
NOLL. 1900. Jahrb. f. wiss. Bot. 34, 457.
NOLL. 1902. Ber. d. bot. Gesell. 20, 403.
[NOLL. 1905. Sitzungsb. d. niederrhein. Gesell. Bonn.]
PFEFFER. 1875. Periodische Bewegungen. Leipzig.
PFEFFER. 1893 a. Die Reizbarkeit der Pflanzen (Verhandl. Gesell. d. Naturforscher).
PFEFFER. 1893 b. Druck- und Arbeitsleistung (Abh. Kgl. Gesell. Leipzig, 20).
[PICCARD. 1904. Jahrb. f. wiss. Bot. 40, 94.]
PINOT. 1829. Annal. Sc. nat., Ire Sér. 17, 94.
[RICHTER. 1902. Function d. Wurzelspitze. Diss. Freiburg.]
SACHS. 1872. Arbeit. bot. Instit. Würzburg, 1, 193.
SACHS. 1873 a. Flora, 56, 321.
SACHS. 1873 b. Arbeit. bot. Instit. Würzburg, 1, 385.
SACHS. 1874. Ibid. 1, 584.
SACHS. 1879. Ibid. 2, 209.
SACHS. 1888. Ibid. 3. Plates.
SCHOBER. 1899. Anschauungen üb. den Geotropismus seit Knight. Hamburg.
DE VRIES. 1880. Landw. Jahrbücher.

LECTURE XXXV

GEOTROPISM. II

HITHERTO we have confined ourselves exclusively to a study of those parts of the plant, such as the chief root or chief shoot, whose position of equilibrium is at right angles to the earth's surface, and which bend back again by growth movements into that position if they be placed in any other. We may describe such organs as *orthotropic*, distinguishing in them two varieties of geotropism, *negative* and *positive*. A casual glance at plants as a whole teaches us, however, that there are many plant organs whose position of equilibrium is other than vertical, and these we term *plagiotropic*, including under that name oblique directions of growth as well as horizontal. It is possible for an orthotropic organ to become plagiotropic under the influence of two forces, i.e. gravity and some other directive force, much in the same way as a body under the influence of two forces acting in different directions obeys neither but takes a new direction easily calculated by reference to the law of the

' parallelogram of forces '. Plagiotropy of this type we will discuss later on ; at present we will confine ourselves to those organs which are *plagiotropic under the influence of gravity only*. An excellent and characteristic example is the horizontally growing rhizome, such as we meet with in *Heleocharis palustris*. The fact that this rhizome grows at a certain depth below the surface demonstrates that one factor which has often a far-reaching effect on the orientation of organs, i.e. light, plays no part in this case (Lecture XXXVI). Since also other directive agents are excluded (Lecture XXXVII) we have only gravity left, and we must assume that the horizontal position taken up by the rhizome has to do with geotropism in some form or another. The correctness of this conception has been demonstrated by ELFVING (1880 a). He planted a subterranean shoot of *Heleocharis* in a vessel filled with loose soil ; the vessel had one wall made of glass so that the direction of the new growth could be studied. When the rhizome is planted in the normal position the new region maintains the same line of growth as the old ; if the apex be bent obliquely upwards or downwards the new region becomes bent sharply back into the horizontal position. If, on the other hand, the axis be *twisted* round in the process of planting so that a *flank* or the *under side* now faces *upwards* no reaction of any kind follows, the rhizome grows straight on horizontally without bending or twisting. From these experiments we may conclude that the rhizome of *Heleocharis* grows, not as in the case of ordinary orthotropic organs, parallel with, but at *right angles* to, the direction of gravity; and yet there is no difference between the sides of the rhizome, which is radial in its structure. The rhizomes of *Scirpus* and *Sparganium* (ELFVING, 1880 a) and also of *Adoxa* and *Circaea* (GOEBEL, 1880) have been shown to behave in the same way, and in all probability the majority of shoots which develop in a horizontal direction in the soil have the same characters (e. g. *Paris*, *Anemone nemorosa*, &c.). In many cases these shoots are the principal shoots of the plants concerned (e.g. *Paris*, *Adoxa*), but lateral branches also exhibit this special form of geotropism which we may term *plagiogeotropism* or *diageotropism*.

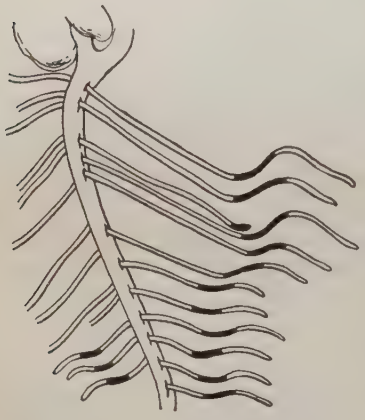


Fig. 138. *Vicia faba*. Chief root with lateral roots, grown in soil behind a glass plate, first in the normal, then in the inverted, and finally once more in the normal position. The increase while in the inverted position is shown in black. After SACHS (1874, p. 605).

The same phenomenon is very obvious in the case of the lateral roots of the first rank, which always form an angle with the rigidly orthotropic and positively geotropic main root. It is quite true that in this case the angle is not always a right angle, it is more often acute, and it is by no means constant in size ; that it is determined by the direction of gravity was shown by SACHS (1874) by simply turning the plant round through 180°. He found that in a short time the new growths made about the same angle with the line of direction of gravity but a totally different one with the line of the chief root, and that after they had been again inverted the original direction of growth was resumed. Fig. 138 illustrates SACHS's experiment, the darkened parts of the lateral roots being those formed whilst the plant was in the inverted position. Again, the lateral roots are strongly radial, for they may be twisted at will round about their long axes without their exhibiting any reaction so long as they remain at the correct angle with regard to the direction of gravity. Curvature follows at once if there be any deviation upwards or downwards from the specific ' limiting angle '.

CZAPEK (1895) has also proved that no reaction takes place if the lateral root is turned at right angles upwards or downwards, but if it be only a little removed from these rest positions a curvature upwards or downwards, according to circumstances, takes place, which ceases when the limiting angle is again reached. This latter position only is, however, the *stable rest position*, the other two positions must be regarded as labile. Rhizomes correspond to lateral roots so far as regards the labile rest positions, but differ from them as to the stable rest position, for that in the case of the root is directed obliquely downwards, while in the rhizome it is horizontal. One would naturally expect that the subaerial lateral organs, e. g. many flowers, lateral branches, would find their stable rest positions when directed obliquely upward; as a matter of fact, branches turn back again to their oblique position if they be forced upwards or downwards. We shall return to this point later, but meanwhile we may note that many flowers, e. g. *Narcissus pseudonarcissus*, exhibit another form of diageotropism (VÖCHTING, 1882). The peduncle is bent over horizontally on the orthotropous scape and if it be taken out of this lie and placed pointing obliquely *upward* or vertical it returns to the horizontal once more; it is remarkable, however, that each reaction *ceases* if the flowers are directed obliquely or directly downwards.

Fig. 139. Formation of the rhizome of *Adoxa moschatellina*. After A. BRAUN (Das Individuum, 1853, Plate II, Fig. 3). *cot*, cotyledon; *K*, axis of the seedling.

While the stable rest position of the orthotropic plant organ is quite constant and is coincident with the perpendicular, we find that plagiotropic rest positions undergo variations not only when *different* organs are considered but also in one particular organ. Thus we meet with very marked differences in a selected example, which are due to internal and external factors. There are especially *two* internal factors, which we cannot always keep distinct: those which depend on the influence of the state of development, the 'ripeness' of the plant, and those which depend on the relationships of the parts to each other and to the whole (correlations). If we study the lateral roots of a bean (*Phaseolus*) which have been grown in uniformly moist soil we find that from above downwards they form successively the following angles with the chief root, viz. 130°, 80°, 80°, 90°, 90°, 65°, 75°, 75°, 40°.

Apart from the *individual* peculiarities of single roots one notices a *decrease* in the size of the angle as the apex of the chief root is approached. Still more remarkable than these differences between lateral roots is the case where a *single* organ in the course of time changes its reaction. Let us study the development of the horizontal rhizome more closely, taking *Adoxa* as our example. The seedling above the level of the cotyledons consists of an orthotropic stem which reaches the light owing to negative geotropism, but later on this stem bends back and buries itself in the soil. The shoot thus exhibits a complete inversion of the normal geotropic reaction, since it now behaves like a plagiotropic secondary root or an *orthotropic* but positively geotropic organ. After it has reached a certain depth the rhizome takes a horizontal direction and produces scale leaves, but it again comes to the surface, acting in a negatively geotropic manner, when the formation of foliage leaves and flowering shoots

begins. After that, the apex of the rhizome once more bends downwards into the earth, then moves horizontally, and then again upwards. These alternations of negative and positive geotropic curvatures with the transitional plagiotropic positions show themselves to be related to the developmental state of the plant, but they are, as we shall see by and by, partly dependent on external factors. The rhizome of *Paris* is originally orthotropic, but when it once becomes plagiotropic (horizontal) it remains so if the external relations be unaltered. In the majority of rhizomes the above-ground flowering shoot does not arise, as in *Adoxa* or *Paris*, as a *lateral* outgrowth from the chief axis, but the end of the chief axis itself comes above ground and becomes orthotropic, while a lateral shoot goes on growing horizontally and continues the rhizome. This phenomenon is seen e. g. in *Heleocharis*, *Scirpus*, *Anemone nemorosa*, and many other plants. In all cases the chief axis grows horizontally in the first years until the flowering period comes on, and, further, the chief axis of the seedling is always originally orthotropic. The first alteration from orthotropy to plagiotropy is due to unknown factors, but the annual or more frequently recurring (*Heleocharis*) changes from plagiotropy to orthotropy and vice versa appear to be bound up with alterations in the morphological and physiological characters of the flowering shoot.

Readjustments of a similar nature, dependent on the degree of maturity of the organ in question, are known to be frequent in the flower axis (VÖCHTING, 1882). The flower bud of *Agapanthus*, for instance, is negatively geotropic, the flower is horizontal, i.e. plagiotropic, the fruit is positively geotropic. The poppy is another well-known example. Its buds exhibit positive geotropic curvatures, which are compensated later by negative geotropism. WIESNER'S (1902) view that the nutation of the poppy bud was due to a special 'weight curving' is not well founded; VÖCHTING'S thesis, which has been described above, has not been met by WIESNER; especially is evidence wanting that the nutation ceases when the weight of the flower is compensated.

Let us now turn to cases where plagiotropism is obviously dependent on influences of correlation.

The influence of the chief axis on the lateral branches is markedly shown when the apex of root or shoot is removed. It has been known for long that when the apical bud of a spruce is removed the plagiotropic lateral shoots of the uppermost whorl become erect and that the strongest of them becomes completely orthotropic and replaces the chief axis. A corresponding experiment has been successfully carried out by SACHS on the root. It thus becomes a pertinent question whether the plagiotropism of the lateral branches is a special character inherent in the branch or whether the rest position in this case is the resultant of two directing forces, so that, though naturally orthotropic, these branches are diverted from the perpendicular by a force emanating from the chief axis. This conception can scarcely be maintained in this form, for BARANETZKY has recently (1901) shown conclusively that the geotropism of the lateral branches is not essentially different from that of the chief axis, and that the actual rest position is conditioned by a special property of the branch. More detailed study will be necessary to appreciate properly what is signified by this peculiarity which all organs possess and which we may term 'autotropism' (PFEFFER, 1893).

VÖCHTING (1882) was the first to show that when a shoot which had suffered geotropic curvature was removed from the unilateral influence of gravity by being placed on a klinostat, the plant attempted to compensate that curvature. The concave side whose growth had been retarded during the curving now elongated more vigorously than the convex side, and hence the shoot became straight once more. It has been already shown that not only in geotropic curvatures of the stem but generally in all cases of induced alteration of form

of this kind a compensatory reaction afterwards occurs which tends to bring about the original form. This compensatory reaction makes itself evident also after a mechanically induced bending of the organ, and it begins, not only after the cause of the bending has been removed, as in the case of geotropism on the klinostat, but also when the organ is subjected to a weight acting continuously and unilaterally. Its effect is, certainly, then only to flatten the curve, not to remove it altogether. Autotropism must also play a part in the straightening of the geotropic over-curvature, which (p. 434) we preferred to regard as due to renewed and converse geotropic activity in the overcurved organ. Autotropism is associated with the increased growth of the side rendered concave by geotropism (Fig. 135). As BARANETZKY showed, an organ, such as a shoot of *Aesculus*, may, after suffering geotropic curvature exhibit on the klinostat several pendulum-like movements, because, just as in the case of geotropism, the autotropism overshoots the mark and is not at once arrested on the attainment of the straight position but causes a new curvature opposed to the first and with it autotropic growth on the opposite side.

Returning to the discussion of branches of trees, BARANETZKY (1901) found that during the evolution from the bud these frequently showed negative geotropism (orthotropism) in a very striking manner, and afterwards took up the oblique rest position. Apart from other factors, which we need not speak of here, autotropism is especially responsible for this supplementary curvature away from the vertical; it causes the axis of the branch to lie in the direction already determined by the relation of the bud to the stem. According to WIESNER (1902) autotropism has nothing to do with this rest position; he believes it due to a special peculiarity of the lateral branch, which we have not as yet referred to, acting in combination with geotropism. This peculiarity is termed *epinasty*, and expresses itself in the effort on the part of the lateral organ to grow more vigorously on the upper than the under side. It must not be assumed, however, that this is an hereditary characteristic of the *morphologically* upper side or that dorsiventrality of the branch has been established; on the other hand, epinasty must be acquired in the course of the life of the individual, if we interpret WIESNER correctly, by the influence of the weight of the branch. It is impossible to pass over WIESNER'S theory without mention, yet the explanation discussed above appears to us to be more worthy of credence.

A closely related problem, and one of great importance, is, what induces the bud to take up a definite direction in space? It may be easily shown, by cultivation on the klinostat, that buds, like lateral roots, form definite angles with their parent axes, due to correlations only, and not at all to external factors. If NOLL'S klinostat theory be correct, then the lateral roots are *not* withdrawn from the influence of gravity on the klinostat; if the chief root be placed in the axis of rotation of the instrument *lessening* of the limiting angle must take place in geo-perception; if placed at right angles to that axis the angle must be increased. The essential experimental data on this subject are as yet non-existent. As may be seen especially clearly in the *root*, the special angle induced in experiments on the klinostat is, as a general rule, *larger* than the limiting angle formed with the co-operation of gravity. In virtue of the internal directive force the lateral roots would lie more horizontally; their position as observed in nature is a resultant. As a matter of fact, we can make the lateral roots approximate to the chief roots and decrease the limiting angle by using a higher speed of rotation (p. 451). The position of lateral shoots is also a resultant of geo- and auto-tropism, and not infrequently it may be noted that as, in the course of development, autotropism decreases negative geotropism predominates, so that axes of inflorescence, for example (e.g. *Aesculus*), come to place themselves quite vertically. Briefly, we may say that the influence of the chief axis on the lateral organ expresses itself in the '*special direction*' and in the angle at which the lateral

organ is laid down but also in increased autotropism. As already mentioned, BARANETZKY (1901) assumes other factors still to account for the rest position of the lateral organs, e.g. the influence of their own weight; into these questions we cannot enter beyond saying that in our opinion a complete explanation of the position of branches is not afforded by including the factors to which BARANETZKY draws attention (NOLL, 1902; [PFEFFER, Phys. II, p. 682]). After these statements it would seem legitimate to inquire whether on the whole there is such a thing as plagiotropism without correlation; the case of horizontal rhizomes might be alluded to, but it appears to us in the highest degree probable that plagiotropism in these structures also is due to correlations. We shall presently learn, however, that plagiotropism may be induced by *external* factors without correlation.

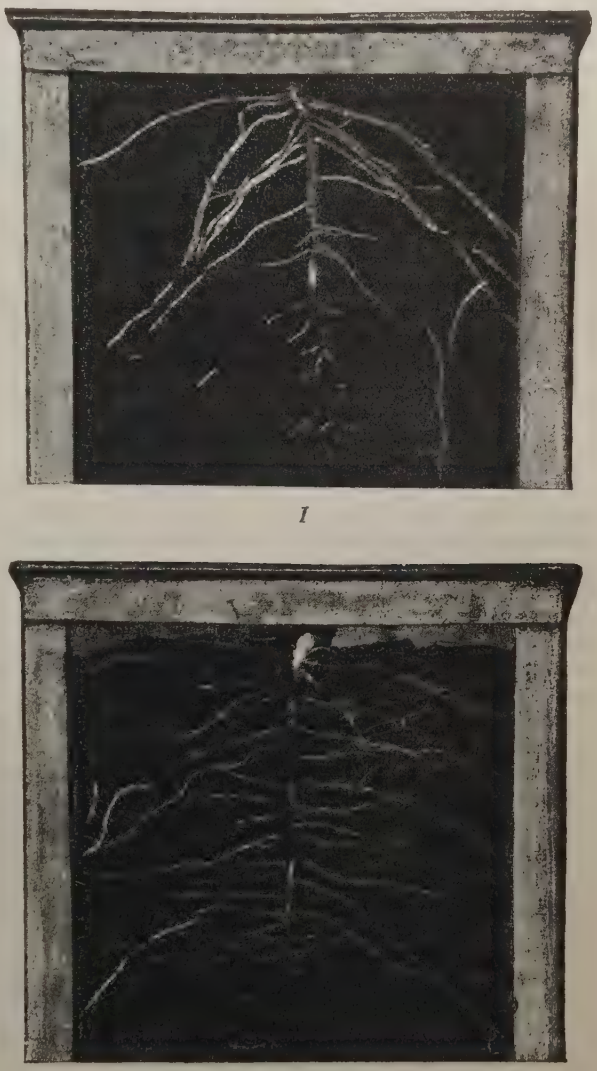
Relationship to the chief axis is not the only factor affecting geotropism; in the shoot itself the capacity for curving is in many cases influenced by correlations. MIEHE (1902) has shown that absence or incapacity of the apex in *Tradescantia* stops or retards the curvature of the basal nodes.

Having now glanced at *internal* factors we have still to consider external influences which alter, often very greatly, the geotropic reaction. The first and most important of these is temperature. SACHS (1874) found that an increase in temperature decreased the angle between lateral and main roots; more remarkable alterations still have been noted by VÖCHTING and more recently by LIDFORS. These authors show that under the influence of a low temperature (a few degrees above zero) normally orthotropic shoots become *plagiotropic*. The shoots of *Senecio vulgaris* in the wild state behave in this way at the beginning of winter, as do also those of *Sinapis arvensis* (VÖCHTING, 1898), *Holosteum umbellatum* (LIDFORS, 1902), and artificially etiolated shoots of the potato (VÖCHTING, 1902). The low temperature operates uniformly on all sides on the shoot, and the plagiotropic rest position cannot, therefore, be regarded as a resultant of two directive forces; gravity is the sole directive agent in this case, but temperature affects the *nature of the response* on the part of the plant, the 'disposition', as one might say. The cases investigated, first by STAHL (1884), of the influence of light on plagiotropic organs may be explained in the same way. A certain intensity of light induces plagiotropic organs to exhibit positive geotropic movements; thus, illuminated rhizomes of *Adoxa* and *Circaea* bury themselves more or less vertically in the soil, not that they turn away from light because they have become negatively heliotropic (Lecture XXXVI), but because they have become positively geotropic, as may be proved by a simple experiment. This alteration in disposition is of the greatest service to the plant, since it prevents the rhizome from growing out of the soil if it lives in suitable surroundings. Other rhizomes certainly react quite differently, and yet quite in accordance with the necessities of the case, for they exhibit negative geotropism when illuminated, and at the same time alter into leafy shoots. Lateral roots behave in the same way as the rhizomes of *Adoxa*, for they, on illumination, make far smaller angles with the chief root than they do when grown in the dark (Fig. 140).

The medium also in which the root grows is of great importance in determining the reaction of the lateral roots; SACHS (1874) found that the rest position of such roots was not the same when cultures were made in soil as when made in water or damp air. The greatest difference appears between the behaviour of the side-root in the soil and in the air; it stands out prominently on the main root (ELFVING, 1880 b). If the root be placed in the soil with its apex directed upwards, a vigorous curving sets in, which almost always results in the reinstitution of the normal direction of growth; if the same experiment be carried out in moist air the downward curvature is only slight and the apices of the roots grow more or less horizontally. Obviously culture in damp air either weakens the geotropic reaction or strengthens autotropism; at all events, by supplying

a stronger centrifugal force (50 g.), by increasing the geotropic excitation, we can induce the same reaction in the chief root in moist air, and re-establish the same orthotropic behaviour as we see in roots grown in the soil and subject to gravity only.

We have now to speak of a fourth external factor which may affect the way in which the organ reacts, the amount of centrifugal force. We know from SACHS's experiments (1874) that the angles formed by secondary roots may be altered by centrifugal force and the roots made to exhibit more nearly orthotropic reactions, and, according to CZAPEK (1895), the same is true of the rhizome. The last-mentioned factor has certainly no effect in nature; it is owing to the combined influence of light, temperature, and the nature of the medium that the rhizome maintains a certain constant depth of position in the soil; we may indeed affirm that its existence would not be possible unless its geotropic reaction could be altered and its 'disposition' be changed.



I

II

Fig. 140. Roots of the seedling of *Phaseolus multiflorus*, grown behind glass. *I*, in diffuse light; *II*, in darkness.

To speak of a 'change of disposition' presupposes of course a 'geotropic disposition' in the plant. We are unable, however, to discover at what point in the chain of processes this makes its appearance. Whether it makes itself evident in connexion with the actual perception, in the structure of the perceiving apparatus (NOLL, 1896), or later on, between the act of perception and the reaction (PFEFFER, 1893; CZAPEK, 1898) is still a disputed point. NOLL's view, viz. that the 'disposition' is governed by the structure of the perceiving apparatus has the great advantage of simplicity and ease of conception, but the assumption of the close relations existing between

perception and reaction is, as we have already said (p. 441), quite arbitrary.

The organs we have hitherto dealt with are radially constructed both as regards their anatomical structure and branching, but from the physiological point of view they only partially assume that position in space which we primarily regard as the normal position of radial organs, viz. the erect ; a large number behave plagiotropically, taking up an oblique or even horizontal position. We should expect *a priori* that all organs with dorsiventral structure would be plagiotropic, and, as a matter of fact, this assumption is correct in by far the majority of cases, for only a few dorsiventral parts, e. g. the shoot of *Vicia faba*, are orthotropic. Dorsiventrality, as previously mentioned, is conditioned very often by external factors, more rarely (as in lateral members, by relations to the chief axis. Among external factors light takes the first place and gravity may combine with it in producing the result. Since many causes co-operate in inducing dorsiventrality it is of course probable that movements of orientation of dorsiventral organs are for the most part conditioned by several factors, the analysis of which is often difficult. Dorsiventral shoots of subterranean rhizomes would form very suitable subjects for the study of movements of orientation, but as such shoots are not at present known we are compelled to have recourse to aerial organs, in which, owing simply to withdrawal of light, functional disturbances take place of so radical a nature that it is impossible to carry out experiments on them. Still some examples are known in which it is possible to exclude the light, when gravity alone makes itself evident. These cases will be sufficient to give us answers to the fundamental questions on the subject.

Let us study first of all the experiments made by FRANK (1870) on horizontal branches of the yew or spruce. The dorsiventrality of these bodies expresses itself externally in the difference in size of the needles on the upper as opposed to the under side, and also in their mode of orientation. If such branches be bent vertically upwards whilst emerging from the bud, or at least during the period when the shoot is still growing, we find that, both in light and in darkness, a curving takes place in them, by means of which they become bent *downwards* into the normal horizontal position, and a corresponding *upward* curvature when they are bent towards the ground. So far the shoots of Coniferae conform entirely with the rhizomes of *Heleocharis*, and possibly they also have labile rest positions directed perpendicularly upwards or downwards. A difference from the radial plagiotropic organs makes itself obvious if we twist round the branch on its long axis so that the under side becomes upper side, the whole branch remaining meanwhile horizontal. It makes efforts at once to invert itself once more so as to make the originally under side face downwards, a result which may be achieved in one of two ways, either by a bending, which will be complete when the branch has described a curve of 180° and turned its apex towards the chief stem, or, more quickly, by retaining the same direction of growth, i.e. radially outwards from the stem, and by inducing a torsion of 180°. The former method, by curving through 180° in one plane, has been observed by SACHS in isolated lateral branches of *Atropa belladonna* ; the latter, torsion through 180°, is illustrated by FRANK'S experiments on branches of Coniferae still attached to the tree. If a dorsiventral branch be fixed after being twisted through only 90°, so that one flank faces upwards, the other downwards, it is obvious that *curvature in one plane* cannot effect a return to the normal position in relation to gravity, and torsions must take place, torsions which have been observed by FRANK (1870) in branches of the second rank after the vertical fixing of branches of the first rank ; the twigs of the second rank then take up a position pointing obliquely upwards, those on the other side pointing obliquely downwards.

Flowers also exhibit this phenomenon, especially dorsiventral or zygo-

morphic forms, as do also leaf-blades, which in most cases are markedly dorsiventral. As an example of a dorsiventral flower which owes its position to the influence of gravity only, and not, as in many other cases, to light, we may take *Aconitum napellus*, which has been studied by NOLL (1885–7). If the inflorescence of this plant be so bent that the terminal portion of the axis with its buds points vertically downwards, and if it be fixed in this position in some appropriate way, after a short time the peduncle becomes curved as shown in Fig. 141. The curvature ceases when the upper part of the peduncle comes to form once more an angle of 30–50° with the direction of gravity, that is, when the hooded sepal has again attained the upper position. If the lie of the flower be regulated by the force of gravity only, this lie (Fig. 141, *II*) must be that of rest. The relation of the flower to the axis of inflorescence has also to be considered, for it is only when the opening of the flower points outwards that insects can visit it and the flower can perform its normal functions. Thus we see that, after this median bending inwards of the peduncle, there follows a complex movement resulting in a torsion of the peduncle and an outward turning of the flower itself. We must leave it undetermined whether, as is to be expected from NOLL's account, a purely geotropic movement is always combined with another autonomous one induced by internal causes (NOLL's exotropism), or whether (SCHWENDENER and KRABBE, 1892) a torsion may take place *without* any median inbending. This much at least is certain that in these orientating movements in flowers of this kind a correlative influence of the axis plays a part; this is especially noticeable in the flowers of the Orchidaceae. The flowers of this order are orientated inversely in the bud, i.e. the labellum is posterior, but owing to a torsion in the ovary during the evolution of the bud they come to assume the normal position. It is possible to prevent this torsion, however, and compel the flower to open in the inverted position if the plant be rotated on a klinostat or if the inflorescence be fixed in the inverted position. The torsion must, therefore, be induced by gravity. If, however the axis be cut off above a flower which has not yet suffered torsion, the flower assumes its normal position by a simple curvature without any torsion, and inclines itself outwards over the top of the cut end of the axis; that is, it performs only the first of the movements which have already been described as occurring in *Aconitum*.

Fig. 141. Inverted flower of *Aconitum napellus* in two stages. After NOLL (1885–7).

Foliage leaves, as we might expect, behave in principle in the same way as do dorsiventral flowers. If the axis be fastened in the inverse position they could regain their geotropic angle and correct orientation of upper and under sides by a simple geotropic axial curvature of the petiole or base of the lamina; they would, however, succeed in carrying out their orientating movements by turning the tips of their blades inwards, though they seldom find space enough available. As a matter of fact, we find them performing the same movements which we have found characteristic of flowers (NOLL, 1887), i.e. a median inward curvature is often followed, as in *Aconitum*, by an exotropic movement, while in other cases, especially leaves with short petioles, the same result is achieved by torsion only. It is impossible to decide at present whether this torsion consists, as NOLL believes, always of two combined movements, or whether it is a single movement (geo-torsion, SCHWENDENER and KRABBE, 1892).

Other torsions also occur, induced by gravity, as in many plagiotropic shoots, e.g. *Philadelphus*. The leaves in the bud of this plant are decussate,

but in the full-grown shoot the leaves are *no longer so*, but all are arranged in two rows along the sides of the branch. This alteration in position is due to *torsion* of the internodes, and since also it takes place in *darkness* (SCHWENDENER and KRABBE, 1892) it must be dependent on gravity, although in the majority of cases such torsions are due to light (Lecture XXXVI).

Since the mechanics of all these torsions have not as yet been fully explained we will not consider them further. Leaves, however, suggest another aspect from which we may study geotropism. Hitherto we have referred all geotropic movements to growth, and have emphasized the fact that they take place only if the organ in question is still in a state of growth or is capable of starting growth anew. In the leaves of many plants (e.g. Leguminosae and Oxalidaceae) movements occur which may take place without any growth and merely by inequality of osmotic pressure on opposite sides of the petiole.

Leaves and petioles do not possess the power throughout their entire extent of altering their state of turgor and so elongating or shortening opposite sides; on the contrary this peculiarity is limited to one special organ, which may be identified externally. Since these organs occur at the base of the leaf-stalk, or (as in pinnate or bipinnate, &c., leaves) at the bases of the pinnae as well, forming relatively short connecting regions between non-motile parts, we may term them *articulations*. For the most part they stand out from parts in the vicinity by reason of their greater thickness, and hence are termed 'cushions' or pulvini. Their anatomical structure is very characteristic and closely related to their function. An examination of the pulvinus of *Phaseolus multiflorus* shows that all the vascular bundles run together in a single axial stand imbedded in parenchyma. The walls of the parenchyma cells are very elastic and extensible, and when in a state of turgor marked differential tissue tensions are induced with the scarcely extensible vascular bundle. If the parenchyma be isolated it elongates very considerably, while the vascular system contracts scarcely at all. As may be easily seen, an increase in turgescence on one side or a decrease on the other, or the occurrence of both phenomena simultaneously, must result in an elongation of one side and contraction of the other, so that the articulation bends. The vascular bundle is of course also bent but undergoes no change in length. Bending of the joint naturally results in a passive movement of the parts of the leaf distal to the articulation.

Movements in the pulvini may be induced by the most varied external stimuli, as also when the leaf is turned upside down by inversion of the stem (SACHS, 1865). In order to bring it back once more to the normal position, for the most part not only are simple curvatures necessary but torsions also occur, which have been even less investigated here than in other cases. In the curving of the joint there is no permanent elongation of the convex side, as PFEFFER (1875) demonstrated by microscopic measurements, and after the inversion of the stem the leaf soon resumes its previous position. From the fact that no growth can be determined, it may be concluded that the curving here is due to an increase of *osmotic* pressure on the convex side and a corresponding diminution on the concave, a conclusion confirmed by the plasmolytic method. Thus HILBURG (1881) found that in the articulation of *Phaseolus* plasmolysis began to appear on the morphological upper side after a geotropic stimulus, only when a 4 per cent. solution of potassium nitrate was used, although before the stimulus a 3 per cent., solution was sufficient; conversely, the osmotic value of the cell contents of the under side, as measured in terms of potassium nitrate, fell from $3\frac{1}{2}$ per cent. to 3 per cent. When the stimulus was permitted to affect the organ for a longer period growth, it is true, finally ensued on the convex side, and when the stem was placed once more in its normal position the leaf was incapable of attaining its original orientation. Although geotropic curvature induced by turgor is limited to the articulations of certain leaves, it is still theoretically of great interest, since it is probable

that the perception of the stimulus of weight is a phenomenon of much wider distribution than the occurrence of geotropic reactions would lead us to suppose. It can scarcely be doubted that perception of the stimulus in very many cases leads to no *visible* result because the capacity for response is wanting in the organs concerned. The leaf articulations are organs of this type which have retained their power of reaction long after growth has ceased in them.

We may now close our discussion of dorsiventral organs and of the external agencies which often to a very marked degree affect their reaction, and conclude this lecture by a reference to twining plants which are distinguished by very special behaviour towards the geotropic stimulus.

In the plants we have been studying hitherto the organs which exhibited movements of orientation had sufficient strength to carry these out in opposition very often to great resistance, evolving considerable energy in the process. We have seen that not infrequently plants are capable of performing far more work than is required for producing movement in the passive regions. A certain rigidity is a fundamental condition of activities of this nature ; when that is absent, the organ, such as a prostrate stem, can never completely raise itself in spite of its possessing a negatively geotropic capacity. There are many plants whose shoots would lie on the ground were they not capable of making use of the rigidity of other plants for their own elevation. Plants of this kind we term climbers. The simplest type of climber is represented by such plants as *Galium aparine*, which, after reaching a certain height by its own unaided efforts, sinks to the ground unless it manages to come in contact with some other plant to which it adheres by means of the prickles which it is provided with. Other climbers exhibit more complicated apparatus designed for the same purpose, such, for example, as the hooks of *Uncaria* or *Strychnos* (Treub, 1882–3 ; Schenck, 1892 ; Ewart, 1898). These organs, however, attach themselves to the support accidentally only, so to speak, and exhibit no active movements designed for the purpose of bringing about attachment, such as are shown by the two great series of tendril-bearers and twiners. From the biological point of view these two series have much in common, since both save themselves the trouble of forming any special skeletal tissue in their axes, and make use of some other rigid structures as a means of supporting the weight of their leaves. In nature these supports are always living or dead plants and hence twiners and tendril-bearers are dependent, quite as much as epiphytes, on other vegetation. Indeed, one might compare them in a sense with parasites, since, if the supporting plants be alive, they shade the light from them and thus inflict injury upon them though, it is true, only indirectly. The movements, however, which the twiners exhibit in their efforts to grasp their supports are so distinct in a *physiological* sense from those exhibited by tendril-bearers, that a combined treatment of the two series is not advisable. At present we will consider *twiners* only, since these plants carry out their movements by means of a *special form of geotropism* (Noll, 1892–1902) and are thereby brought into close relation to the subject we have just been discussing.

Twiners grow round the stem of the supporting plant in a spiral manner, and since those spirals lie close to and exert pressure on the support, and since, further, the twining stems are frequently rough, the attachment is thus rendered very secure and slipping away from the support is practically precluded. On closer examination of a twiner, e. g. *Calystegia*, which begins to develop in spring, we note that its shoots are at first strongly orthotropic and hold themselves erect in virtue of a certain rigidity which they possess. After reaching a certain height, however, the apex of the shoot bends over, and that too in consequence of an active movement, not merely in consequence of the weight of the apex, and takes up an almost horizontal or plagiotropic position. At the same moment a special type of movement begins to make itself apparent, which may be termed

a *revolving motion*, since the horizontal apex rotates round the fixed vertical basal region like the hand of a clock. This movement continues so long as the shoot remains capable of growth and as a rule is always in one definite direction. In the majority of cases the revolution, looked at from above, is *opposite* in direction to the clock-hand, i.e. to the *left*; the direction of motion in the hop and honeysuckle is, however, on the contrary, to the *right*, while alternating left and right revolutions have been observed in *Bowiea volubilis* and *Loasa lateritia*. Plants which revolve to the left (Fig. 142) twine round their supports to the left, and those which revolve to the right twine to the right (Fig. 143). Since there is obviously a close connexion between the mode of twining and the revolving motion it will be necessary to study this new type of movement somewhat more closely.

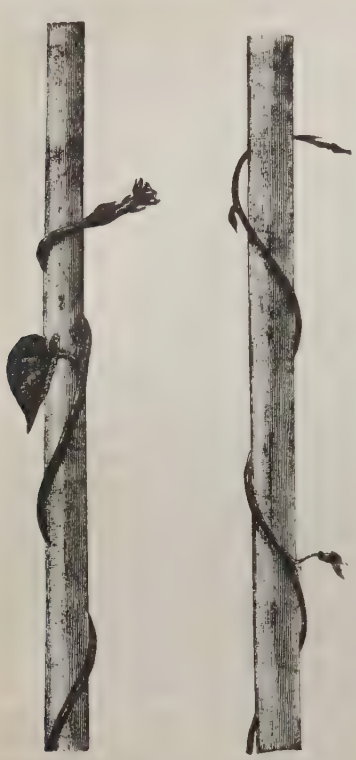


Fig. 142. Fig. 143.

Fig. 142. Left-handed twining shoot of *Pharbitis*. Fig. 143. Right-handed twining shoot of *Myrsiphyllum asparagoides*. From the Bonn Textbook.

The rotatory movement may be most easily explained by means of a simple model. Fasten a thick-walled caoutchouc tube by its base to a vertical peg, and place in its apex a piece of lead tubing sufficiently heavy to bend the tube over into the horizontal position, and rotate with the hand the apex of the tube in the reverse direction to that of the clock-hand (Fig. 144). If a small indicator, say a needle, be fastened to the end of the lead piping, pointing downward at the commencement of the movement, we find that this needle points to the left after a quarter of a revolution (looked at from without), after half a revolution it points upwards, and so on; but one also sees that the torsion of the horizontal part on its long axis, as seen from without, is *in the direction* of the clock-hand. One may easily convince oneself of the fact that the torsion is in the reverse direction to the rotation by drawing a line on the long axis of the tube which is seen to revolve round the horizontal part of the tube. *The advancing surface of the tube thus changes moment by moment as the revolution proceeds.* If we desire the movement to be carried out in such a way that the *same* surface always lies in front, we must hold the apex of the tube firmly during the revolution, but if one revolution be completed under such conditions a torsion ensues in the lower end of the tube, a torsion which is immediately undone as soon as the end is released, in consequence of which there develops at once the same torsion in the apical region as we have previously noted during the complete revolution. If now we mark on the horizontal and the bent region of a shoot of *Calystegia* one definite surface, by painting a line on it with indian-ink, we may note that, just as in the model, this line twists round in the

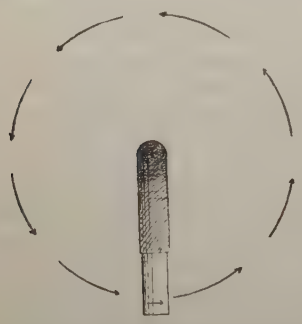


Fig. 144. Model to illustrate twining.

direction of the hands of the clock. When the rotating end of the shoot has passed through 360° in a direction contrary to the clock-hand, the end of the inked line has twisted round through 360° in a direction conformable to the motion of the clock-hand; in other words, a complete revolution in one direction is accompanied by a complete twisting in the other, and in the end the shoot is just as it was in the beginning and no torsion of any kind is observable in the vertical part of the shoot.

This rotatory movement has for long been considered autonomous, resulting from the operation of internal factors, and, as a matter of fact, we shall presently become acquainted with similar movements which are autonomous. The revolutions of twining plants are, however, conditioned by *gravity only*; in other words, they are *geotropic*, but here we have to deal not with increased growth on the upper or under surfaces of the organ but on its *flanks*; in plants which twine to the left it is the *right* flank (seen from above) that grows more vigorously than the left, and vice versa. The whole shoot, however, does not exhibit such 'lateral geotropic' reaction; on the contrary, it is limited to the intermediate region between the erect and the horizontal portions of the shoot, for the erect region is simply *negatively* geotropic while the horizontal region is *diageotropic*. As soon, therefore, as the right flank of the bent portion is induced to grow more rapidly by the stimulus of gravity the horizontal region begins to rotate, and, in order to avoid torsion in the basal region, it must, as we have seen, twist on its axis and turn another surface towards the right flank. Thus new surfaces directed to the right are successively subjected to the lateral geotropic stimulus.

In proof of the geotropic nature of these rotating movements we may advance the following evidence.

1. These movements, as SCHWENDENER (1881) first pointed out, and as BARANETZKY (1883) afterwards confirmed, cease to be exhibited on the klinostat, the axis straightens and performs only certain irregular oscillatory movements, obviously due to internal causes (autonomous nutations, Lecture XLI). If the revolving movements are to be correlated with these nutations and also termed autonomous (WORTMANN, 1886), and if it be said that gravity merely influences their *direction*, then all geotropic movements must be considered autonomous, since such nutations are universally distributed. As a matter of fact, C. DARWIN (1881) attempted to show that all movements in response to stimulus were modified nutations, a contention which has not been verified.

2. If we place the tip of the revolving stem against a stick, it stops the movement and a tension arises in the shoot, because the right flank proceeds to elongate more rapidly than the left. If it were the fact that after a time, for *inherent* reasons, *another*, e. g. the under, surface proceeded to grow more vigorously, the apex would be *lifted up*, the flank lying against the stick must grow more rapidly and the tension would be counteracted. But nothing of the kind is to be seen; indeed NOLL (1885) draws attention to the fact that in his experiments the tensions were maintained for days.

3. If we twist a revolving shoot of *Calystegia* through 180° and fix it so that what was the upper surface is now the lower, and so that the previously convex side now faces to the left and the concave side to the right, the curve becomes flatter, and finally a new curving commences in the reverse direction. The *rapid* reaction of a surface which is suddenly brought into the proper position to receive the stimulus, and the absence of any *after-effect* in the previously stimulated surface, must at least awake in us the suspicion that we have to deal here with other conditions than those of ordinary orthogeotropism.

The cause and the mechanism of the revolving movement ought now to be generally obvious. We need not go into the complications which arise when irregularities of movement occur in the apex of the shoot, but merely note

that the apical region does not always lie horizontally. We have yet to explain the significance of this rotatory movement in the *twining*.

Twining commences if we place a more or less upright support beside the greatly overhanging apex of a revolving shoot. The support is enclosed at first by slack, very flat spirals, which gradually become steeper. This elevation of the apex takes place in obedience to negative geotropism, and if the support be subsequently removed—other conditions being suitable—the spirals are straightened out and the shoot appears twisted; if the support be not withdrawn the successive spirals become tighter, compressing the support. The characteristic of the climbing plant lies in the gradual transition from the diageotropic horizontally placed apex to the negatively geotropic base through the laterally geotropic overhanging region. The twining arises from a combination of the revolving movement and negative geotropism, the support rendering the otherwise inevitable straightening of the shoot impossible. The *twining movements* take place, however, *without* any support, just as they do when it is present, in proof of which we may cite the free twinings which many shoots exhibit when they have grown beyond their supports or when isolated and placed in water (SACHS, 1882); far too little is known, however, about the origin of such movements to justify us in drawing any conclusions from them. If *they* also arise as a result of revolution and negative geotropism combined, the capacity for growth must disappear very much earlier in them than in normal shoots. Ordinarily speaking *no* such free twining is observable at the free ends of the shoots of climbers; on the contrary, after one complete revolution the apex remains *essentially* unchanged, exhibiting in specially simple cases a curving almost in one plane. WORTMANN (1886) and, earlier, DE VRIES (1873) tied a fine silk thread round the apex of the shoot, placed it over a pulley, and supported the weight of the overhanging shoot by a small compensating weight. Under such conditions according to WORTMANN, the simple revolving movement, such as one sees in the free apex, no longer occurs, but the shoot begins to exhibit twining, the spirals being at first flat but afterwards becoming gradually steeper. The support acts often like the weight in this case, i.e. it prevents the shoot from sinking down and permits negative geotropism to have its legitimate effect. Whether the silk thread *merely* supports the weight of the apex, as WORTMANN affirms, *without altering* the movements in any way cannot be decided; it would appear to us at least as if the thread must hinder the torsion of the apex on its axis.

A detailed analysis of the twining phenomena is still unfortunately unavailable, and by no means all investigators hold the view that twining may be accounted for by *revolving movement and negative geotropism alone*. SCHWENDENER (1881), for example, postulates as essential a so-called 'grasping movement' in addition to these factors. The apex of the twining shoot must come in contact with the support from time to time, and in consequence of the tensions set up thereby the incurvings of the shoot becomes transformed into *permanent* twinings. It is impossible to deal with this question here, for details of which reference must be made to the literature. (In addition to the authors already cited see also AMBRONN, NOLL, and KOLKWITZ.) Similarly, we must omit any discussion of the *torsions* which are of such frequent occurrence in twining stems and which have been so variously interpreted.

Instead of discussing difficult problems like these we prefer in conclusion to direct attention to certain important phenomena which strongly support our conception of the co-operation of geotropism in twining movements. If we turn a twining shoot of *Calystegia* upside down some of the youngest spirals first of all begin to open out, then to bend backwards, and proceed to curve *upwards* in the original direction, viz. to the left. The undoing of the spirals already formed plainly shows that the lateral geotropism is not yet obliterated in them, but when

inverted it is the inner flanks of the spirals which exhibit increased growth, hence bringing about a straightening of them.

If geotropism be so active a factor in the twining phenomenon we are led to conclude that twiners can regularly embrace only such supports as are more or less erect. Indeed the longer the overhanging part of the shoot is, the more will the inclined supports be seized, as one may observe especially in plants like the hop.

The clasping of the support at right angles is compensated for by a peculiarity of the climber, which we have hitherto not drawn attention to. As in the case of many etiolated shoots, twiners have inordinately long internodes with at first very small leaves, and there are many advantages in these leaves not reaching their full size until after the stem which bears them has obtained a firm hold on the support, first, because the weight of the stem would be appreciably increased, and, secondly, because the interruptions in the rotation which would result from the striking of large leaves against the support are thereby avoided. The advantages of possessing long internodes require no special explanation, so that we may at this point close our very fragmentary observations on geotropism in general and twining in particular.

Bibliography to Lecture XXXV.

Ambronn. 1884. Berichte math-phys. Kl., Gesell. d. Wiss. Leipzig.
Baranetzky. 1901. Flora, 89, 138.
Baranetzky. 1883. Mém. de l'Acad. de St. Pétersbourg, Ser. 2, 31.
Czapek. 1895. Sitzungsber. Wiener Akad. 104, 1197.
Czapek. 1898. Jahrb. f. wiss. Bot. 32, 175.
Darwin. 1881. Das Bewegungsvermögen d. Pflanze. German edition by Carus, Stuttgart.
Elfving. 1880 a. Arb. d. bot. Instit. Würzburg, 2, 489.
Elfving. 1880 b. Acta Soc. Fenn. 12.
Ewart. 1898. Annales Jard. bot. Buitenzorg, 15, 187.
Frank. 1870. Die natürl. wagerechte Richtung von Pflanzenteilen. Leipzig.
Goebel. 1880. Bot. Ztg. 38, 790.
Hilburg. 1881. Unters. bot. Instit. Tübingen, 1, 23.
Kolkwitz. 1895. Ber. d. bot. Gesell. 13, 495.
Lidfors. 1902. Jahrb. f. wiss. Bot. 38, 343.
Miehe. 1902. Jahrb. f. wiss. Bot. 37, 527.
Noll. 1885. Bot. Ztg. 43, 664.
Noll. 1885–7. Arb. bot. Inst. Würzburg, 3, 189 and 315.
Noll. 1892. Heterogene Induktion. Leipzig.
Noll. 1896. Das Sinnesleben d. Pflanzen. (Ber. Senkenberg. Gesell. Frankfurt.)
Noll. 1901. Sitzungsber. niederrhein. Gesell. Bonn.
Noll. 1902. Ber. d. bot. Gesell. 20, 403.
Pfeffer. 1875. Die periodischen Bewegungen. Leipzig.
Pfeffer. 1893. Die Reizbarkeit d. Pflanzen. Leipzig.
Sachs. 1865. Experimentalphysiologie. Leipzig.
Sachs. 1874. Arb. bot. Inst. Würzburg, 1, 584.
Sachs. 1879. Ibid. 2, 226.
Sachs. 1882. Ibid. 2, 719.
Schenck, H. 1892. Beitr. z. Biologie u. Anatomie der Lianen. Jena.
Schwendener. 1881. Sitzungsber. Berlin. Akad. 1077.
Schwendener. 1886. Ibid. 663.
Schwendener and Krabbe. 1892. Abhandl. Berlin. Akad.
Stahl. 1884. Ber. d. bot. Gesell. 2, 383.
Treub. 1882–3. Annales Jard. bot. Buitenzorg, 3.
Vöchting. 1882. Die Bewegungen d. Blüten u. Früchte. Bonn.
Vöchting. 1898. Ber. d. bot. Gesell. 16, 37.
Vöchting. 1902. Bot. Ztg. 60, 87.
De Vries. 1873. Arb. bot. Inst. Würzburg, 1, 317.
Wiesner. 1902. Sitzungsber. Wiener Akad. 111, 733.
Wortmann. 1886. Bot. Ztg. 44, 273; 601; 617.

LECTURE XXXVI

HELIOTROPISM

THE distribution of light also induces the plant to take up a certain orientation in space ; one may speak of *phototropism* just as we have of geotropism, or—since in nature the sun is the only source of light which need be taken account of—we may term it more particularly *heliotropism*. There are many analogies between geotropism and heliotropism, of which the following are the chief :—

1. Just as geotropic curvature arises only from the unilateral influence of gravity, so heliotropic curvatures are induced only by *unilateral* incidence of light. The same apparatus which withdraws the plant from the *unilateral* action of gravity, the klinostat, enables us also to prevent heliotropic curvatures by rotating the plant in its normal position round a vertical axis. The same effect may naturally be attained by revolving the light round the plant or if we permit the light to stream on the plant on all sides equally (e. g. diffuse daylight).

2. The different organs of the plant react in different ways to unilateral illumination. *Orthotropic* organs arrange themselves parallel with the path of the incident ray, and grow either in a *positively* heliotropic manner towards the source of light or *negatively* away from it. *Plagiotropic* organs, on the other hand, arrange themselves so as to make a definite angle with the incident rays. Organs which are orthotropic as regards gravity are almost always orthotropic as regards light, and the plagiotropic behaviour of other organs shows itself also in heliotropism just as in geotropism. For the most part each organ possesses a power of appreciation of the stimulus of light *and* of gravity, and if the reactions to these two forces do not annul each other, a positively geotropic organ must be negatively heliotropic and vice versa ; that this is the case for the most part experience teaches us.

From the analogies which exist between them the conclusion has been drawn that geotropism and heliotropism are closely related phenomena, and hence they have frequently been considered in conjunction. The reason for treating them separately here is that, as will be shown later, there are great differences between them.

As we have noted above, in nature light and gravity act for the most part simultaneously on the plant, and we must assume without deeper knowledge that the one interferes with and not infrequently neutralizes the other. If, however, both affect the plant in the *same* way, then one could not learn anything as to the one component which is of interest to us at the present moment, viz. heliotropism, unless it were possible to exclude geotropism altogether. This, however, can be done, for we may cause the plant to rotate in the vertical plane on a klinostat and arrange it so that it is illuminated on one side only by placing the axis of the klinostat parallel with a window. Experience has shown that so complicated an experiment is by no means essential, since the effect of light often makes itself so prominent that geotropism would appear to be absent altogether. In the present lecture we will confine ourselves to the establishment of this fact—one of the highest importance—and consider the combined action of light and gravity later (Lecture XXXVII).

The fundamental phenomenon of heliotropism may be seen in any plant which has been cultivated in a room at a distance from the window. Let us consider a seedling of *Sinapis alba* growing in a culture solution (Fig. 145). It will be seen that in a very short time the stem bends towards the window until its axis is approximately parallel with the line of incidence of the light ; it is positively heliotropic and orthotropic. The root behaves in the converse way, it is orthotropic but negatively heliotropic. The leaves on the other

hand take up their positions partly passively, owing to the bending of the stem, partly actively, so that their upper surfaces are exposed to the light, i. e. they are plagioheliotropic. Positive heliotropism is a common phenomenon among the shoots of the higher plants, especially in the seedling condition; it occurs in orthotropic leaves also, such as one meets with in the seedlings of many Monocotyledons. It is by no means limited to green plant organs, for it is to be met with in many Fungi. Thus the stalks of the fruits of *Peziza fukeliana* and of *Coprinus*, the perithecia of many Pyrenomycetes and the unicellular sporangiophores of *Phycomyces*, *Mucor*, and *Pilobolus* all bend towards the light. A certain small number of roots also, e.g. those of *Allium sativum*, are positively heliotropic.

We are not nearly so well acquainted with the process of positive heliotropic curvature as we are with geotropic movements. The little we do know tends to show that heliotropic does not differ fundamentally from geotropic curvature. The comparison comes out first of all in the fact that—apart from leaf articulations—the curvature is due to *unequal growth* of opposite sides, and that apparently growth is retarded on the concave side and accelerated on the convex; at the same time the median zone, frequently at least, grows on at a uniform rate during the curving. Generally speaking the first beginning of the curving is manifested at the zone of most vigorous growth in heliotropism also, which then proceeds basipetally, and becomes stationary in the last segment capable of growth, while the apex straightens again. We shall meet with exceptions to this behaviour later.

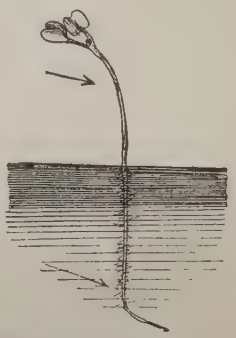


Fig. 145. Seedling of *Sinapis alba*. The arrows mark the incidence of the light. The stem is positively, the root negatively heliotropic. After SACHS (1882).

In days gone by positive heliotropic curvature was explained in a very simple and to a certain extent purely mechanical way (DE CANDOLLE, 1832). The explanation was based on the fact that growth in length in many organs was retarded by illumination and accelerated by shading. Hence, if an organ is more brilliantly illuminated on one side than on the other the former must remain shorter and become in consequence concave, and so the organ must exhibit positive heliotropic curvature. This hypothesis was, however, overthrown by closer study of negatively heliotropic organs. Negative heliotropism occurs in many terrestrial roots, but most of all in aerial roots, in many tendrils, the hypocotyl of the mistletoe, and, among unicellular structures, in the roothairs of ferns and liverworts, &c. These organs all grow more rapidly in darkness than in light, and on being illuminated on one side ought to exhibit the same curving as is exhibited by positively heliotropic organs. Since they do not do so, and since the illuminated side grows more rapidly and becomes convex, we may fairly conclude that heliotropic curvature does not arise directly from a difference in the amount of illumination on opposite sides of the organ. As was the case in geotropic curvature, so here also we have to do with a *uniform reaction of the entire plant* to an external stimulus.

In order to prove this view of the case completely it has to be shown that one and the same organ, may, according to the conditions, react positively or negatively. Evidence of the most convincing character to this effect has been brought forward by BERTHOLD (1882) in certain marine Algae, which were positively heliotropic in weak light and negatively heliotropic in brighter light. STAHL (1880) has observed the same phenomenon in *Vaucheria*, and OLTMANNS (1897) has advanced exact experimental proof of a similar nature in the case of *Phycomyces*, by allowing the fungus to grow at varying distances from an electric arc lamp. Half an hour after the commencement of the experiment

he found the sporangiophores exhibiting *positive* phototropism at a distance of 80 cm. from the source of light (the light intensity being the equivalent of 8,000 Hefner lamps); at a distance of 20–30 cm., on the other hand, with a light intensity equal to about 100,000 Hefner lamps, they showed *negative* curvature. As a result of these experiments it is obvious that there is a certain intermediate light intensity which has no phototropic effect at all. Indeed OLTMANNS' experiments have proved that sporangiophores remain *erect* if they be farther than 30 cm. from and nearer than 80 cm. to the source of light. An hour after the beginning of the experiment the negative curvatures had become still more apparent, but at a distance of 60–70 cm. no phototropic reaction was induced.

Each organism may, therefore, be found in one of *three* different conditions determined by light intensity, viz. (1) a condition of positive heliotropism; (2) a condition of indifference; (3) a condition of negative heliotropism.

Before considering OLTMANNS' experiments further in detail it must be first of all emphasized that the heliotropic effect depends essentially on a factor we have not as yet noticed, viz. the *intensity of the light*. WIESNER, some time before (1878), studied the influence of the intensity of light on heliotropic curvature by illuminating, unilaterally, etiolated seedlings by means of a gas flame of constant but very limited intensity. He placed the plants at different measured distances from the source of light and calculated the intensity of the light which fell on the exposed face of the plant. He noted further the beginning of the curvature and the inclination from the vertical which each had attained after four hours. He found that in the case of *Vicia sativa*, with a light intensity equal approximately to a half spermaceti candle power, curvature was most quickly induced (after seventy minutes), and reached its maximum (to the horizontal position). The increase or decrease of the light afforded a measure both of the time before reaction commenced and of the angle of inclination, conforming with a decrease of the heliotropic stimulus. Quite close approximation to the flame induced no bending nor even growth, a result which at first does not appear to correspond with the results obtained by OLTMANNS with *Phycomyces*. Closer examination shows that the intensity of the light in WIESNER'S experiments was very low (about 200 candles), while OLTMANNS, both in respect of *Phycomyces* and of the etiolated seedlings of cress and barley, first saw curvatures taking place when the intensity of the light was the equivalent of from 10,000 to 15,000, likewise 400,000–500,000 and 500,000–600,000 Hefner lamps. In WIESNER'S experiments injurious effects must have been induced by the gas flame, and this exhibits itself in the fact that WIESNER found growth ceased in the indifferent position, which was not the case in OLTMANNS' experiments. WIESNER attempted to determine the lower limits of light intensity also, but in place of his results we will quote the more recent experiments of his pupil FIGDOR (1893). This latter investigator found that light intensity approximately equal to 0·002 of normal candle power was no longer sufficient to induce any heliotropic curvature in the seedlings of *Vicia sativa*. At the same time other plants behaved in a different manner; *Lepidium sativum*, for instance, still exhibited heliotropism with light of 0·0003 candle power, and in *Raphanus sativus* light of 0·016 candle power had no longer power to induce heliotropic curvature.

Summarizing these results we may say that there is a certain minimum intensity of light at which heliotropic curvature commences but that this liminal stimulus varies in different plants; and that there is also a certain light intensity at which the curvature takes place in the shortest time and exhibits the greatest angle; and, thirdly, a light intensity at which no heliotropic curvature follows, an intensity which OLTMANNS regards as the best for the organism or organ in question, i. e. the *optimum* illumination for its general welfare, while the second intensity referred to above is to be regarded as the

optimum for positive heliotropic curvature. Finally, by further increase in light intensity, we reach a new liminal value, viz. that for negative curvature. Both negative and positive curvature have for their object the placing of the plant in the position of optimum light intensity. It may be possible to establish, at least for some plant organs, that all light intensities which produce negative curvature do not act equally, but that as the intensity increases the negative curvature also at first increases and later decreases. We would thus have a curve with three zeros, regarding the abscissae as indicating light intensities and the ordinates as representing the amount of reaction (relatively their reciprocals). Such a curve is illustrated at Fig. 146.

As OLTMANNS has pointed out (1892) the whole curve is not given by every organism; at the most we may expect that shade-loving plants (amongst which we may include *Phycomyces*) will exhibit *both* positive and negative halves of the curve. Photophilous plants, on the other hand, demand so much light that we must be satisfied if we can prove generally that they are no longer positively heliotropic when the light reaches a certain intensity, for we are unable to recognize negative heliotropic curvature in them. On the other hand, roots perhaps show only the negative section of the curve, for they are negatively heliotropic only when the light is very bright, and to all lower intensities they are quite indifferent.

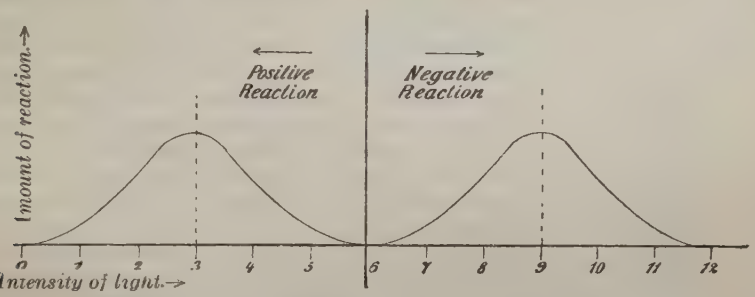


Fig. 146. Diagrammatic curve, to show the relation of heliotropic reaction to light intensity.

The problem of heliotropic reaction is complicated by the fact that the cardinal points of the curve (the zeros and maxima) are not constant in the case of any selected plant. They vary both according to external and internal conditions. Amongst the former *light* itself is by far the most important. OLTMANNS (1892) found that the longer *Phycomyces* was illuminated the more its adaptability to light increased, for sporangiophores which were negatively heliotropic at the commencement of the experiment became, after a few hours, indifferent or even positively heliotropic. One culture, which had been strongly illuminated for ten hours, showed next day a strong tendency to bend over in the reverse direction. Further, the previous illumination has a great influence on the initiation of the positive curvature, for it is known that the commencement of the reaction takes place at a much lower intensity in etiolated plants than in those which have been grown in light. Among internal factors which influence light adaptability the *age* of the organ has to be considered. *Young* sporangiophores of *Phycomyces* are more adaptable than *old* ones; indeed, the latter respond more readily in negative manner. The heliotropic adaptability of certain flowers has long been a familiar fact; such flowers are positively heliotropic when young but react negatively after being fertilized. e.g. *Linaria cymbalaria* (HOFMEISTER, 1867) and many others (WIESNER, 1880; HANSGIRG, 1890). In these cases the power of adjustment is obviously of service to the plant, for flowers exposed to light will be more readily seen

and visited by insects for purposes of cross-pollination, whilst it is preferable that the fruits should be sheltered from sunlight. Without proving it in detail it may be generally accepted that heliotropic movements have in each case some definite purpose to fulfil.

So far we have considered only *unilateral* illumination. In nature such a condition of affairs is best seen in the case of plants which grow against a wall or under trees or bushes, and in these heliotropic curvatures are specially observable. At the same time freely exposed plants may be expected to exhibit heliotropic curvatures also, since, as the sun circles round them, the north side of the plant will receive markedly less light than the south side (at least in our latitudes). We might expect that such plants would curve towards the brightest light, i. e. towards the south, or that they would take up different positions during the course of the day, following the course of the sun. WIESNER (1901) has, as a matter of fact, shown experimentally that heliotropic curvatures may appear with sufficient rapidity to enable the plants to follow the sun's course in the heavens. When he allowed seedlings of *Vicia sativa* to revolve once in twenty-four hours, the plants being unilaterally illuminated through a slit in an apparatus designed for the purpose, he found that, notwithstanding the rotation, the seedlings bent continuously towards the slit. In nature, however, we find that the plant neither follows the sun's course nor does it bend towards the south. The reason for this is that even direct sunlight is too bright to bring about heliotropic curvature; only diffuse, not direct, sunlight has the power of inducing heliotropic movements, as WIESNER has again and again proved.

Fig. 147. Heliotropic curvature exhibited by the leaves of *Polygonum fagopyrum*. The arrows indicate the direction of incidence of the light rays. After FRANK (Lehrbuch der Botanik).

It was already noted that in WIESNER'S experiments (1878) the apices of the seedlings arranged themselves exactly parallel with the direction of the incident ray only when the intensity of the light reached a certain degree, and so, as we may assume, attained the position of heliotropic equilibrium; in other cases they formed larger or smaller angles with the horizontal, and we cannot say whether this deviation from the position of equilibrium is due to autotropism or whether it is really the resultant of the combined action of geotropic and heliotropic stimuli. Both views are possible, though the former seems to us to be the more probable; this at least is certain, that autotropism acts in opposition to heliotropism, and can be neutralized only when the stimulus reaches a definite amount. There is a third possibility, the structure in question may not be orthotropic but plagiotropic. This is certainly not the case with the principal axis, but many lateral branches, although radial in structure, are plagiotropic. Since, however, there are no investigations recorded on this subject which enable us to settle the question, we may turn at once to dorsiventral organs whose plagiotropism undoubtedly bears a definite relation to light. The leaves of the higher plants will serve as an illustration of this relationship.

Foliage leaves have, in the great majority of cases, a quite distinct dorsiventral structure, since the upper side normally receives a greater amount of light than the lower. The light position of the leaf-blade demonstrates itself very clearly when we examine a simple case, where *diffuse* light plays upon *one* side only or at least affects one side *more* than the other. If the strongest diffuse light is directly overhead, as is the case in a wood (WIESNER, 1899), the leaf-surfaces are exactly horizontal. If, on the other hand, it be directed horizontally, the leaves must behave very differently according to their positions on the axis, if they are to

get the maximum amount of light. In general, however, we may recognize four principal positions which the leaf may occupy with regard to a laterally placed source of light; it may be illuminated from the front, from behind, or from either flank. The movements exhibited by the leaf have been described by FRANK (1870) in the following terms:—'Leaves facing the light curve until their upper sides are convex, they then bend downwards so as to expose their surfaces to the light. The same relative position is obtained by leaves on the shaded side by the upper surface becoming concave and bending upwards. Lateral leaves obviously cannot attain the optimum light position by bending in one place, and hence they exhibit torsions which place the laminae in a vertical position, one margin pointing upwards, the other pointing downwards, the surface being orientated at right angles to the light.' These phenomena are illustrated at Fig. 147. If, finally, the source of light be beneath the leaf, a torsion of 180° takes place, so that the normal orientation of upper and under surfaces with reference to light is again attained. This latter case is illustrated by the leaves of 'weeping trees', and it may be induced experimentally in other plants by artificial illumination from the under side, e. g. by aid of a mirror.

The region of heliotropic movement entirely depends on the structure and distribution of growth in the leaf. Naturally, the basal parts of the leaf are those most concerned, whether the base be in the form of a petiole or merely part of the lamina. Leaves with long petioles are especially worthy of observation, since in such cases the angle which appears between the blade and the petiole shows that the two component parts of the leaf do not react in the same manner to the external stimulus, the petiole curving more into the erect, the lamina more into the horizontal position. We shall find later on, however, that the movement of the petiole is not independent but is initiated by the lamina. If the leaf be branched, heliotropic movements are carried out as a general rule by the individual *leaflets*, at all events after they reach a certain age.

Heliotropic *curvatures* are readily recognized as growth phenomena, but the mechanics of the *torsions* have not as yet been fully explained. It has long been believed that these torsions were occasioned only by the action of a series of external factors, such as light, gravity, weight of the organ, which individually led to curvatures, but in combination induced torsions, but later investigations have shown that torsions might appear when light only was the functional external factor. Thus VÖCHTING (1888) has clearly demonstrated torsions of purely photic origin in the leaves of the Malvaceae, and SCHWENDENER and KRABBE (1892) have proved that such torsions do not occur in the great majority of leaves when experimentally examined with the aid of the klinostat, although they certainly made their appearance in the peduncles of certain flowers. We cannot enter here into a discussion of the possible reasons which may be advanced to account for the frequent non-occurrence of torsions when plants are subjected to unilateral illumination on the klinostat; we need only note that, generally speaking, dorsiventral organs, when placed on the klinostat, carry out special movements, such as the remarkable epinastic curvatures of leaves (compare p. 449), and that it has not as yet been shown whether these organs are stimulated geotropically on the klinostat or not (compare p. 438). Although, among external factors, light alone is sufficient to induce torsions, still there is one internal factor which might co-operate (exotropy, NOLL, 1885–7), but as to this we are not in a position to give a final decision. If the torsions cannot generally be regarded as due to the combination of two curvatures (SCHWENDENER and KRABBE, 1892) we are completely in the dark as to.the mechanics of their production. One fact only seems to be established, viz. that, *in general*, growth also plays a part in the production of these torsions.

Certainly that is not so in all cases. A not inconsiderable number of leaves, as we have already seen, are able to carry out geotropic curvatures, by alteration of turgor only, in special articulations, and without growth, and these articulations are capable also of exhibiting heliotropic responses. In *Robinia pseudacacia*, for instance, the main petiole and its articulation remain almost stationary, while the leaflets exhibit continuous movements during the day. In light of weak intensity the leaflets take up the same positions that we have seen them assume in movements occasioned by growth; if we imagine, for the sake of simplicity, that the chief petiole stands horizontally and that the strongest diffuse light falls on it from above, we shall find that all the leaflets arrange themselves horizontally also; if, however, the light strikes the leaf in front in the same direction as that in which the petiole lies, the leaflets twist round at their articulations through an angle of 90°, so that they may adjust their surfaces vertically at right angles to the incident ray. If a bright light, e.g. direct sun's rays, be allowed to fall on the leaf from above, we find that an entirely new phenomenon makes its appearance, for the horizontally placed leaflets elevate themselves, and each forms with its opposite neighbour an angle of 90° or less, instead of 180°, so that the upper surfaces approximate. No matter what be their position the leaflets execute movements, so that they make with the incident ray a very small angle or even turn their edges to it. We have thus two extreme positions to consider, a surface position adapted to feeble light and a profile position for strong light. These movements must have some purpose, whether over-bright light is injurious or whether the object is only to avoid excessive transpiration. The intermediate positions are especially purposeful, since they obviously render possible the absorption of an amount of light adapted to its intensity.

Leaves which have no articulations are, however, unable to alter their space relationships continuously, hence they assume, during their growing period, a fixed light position, which is determined not by *direct sunlight* but by the *strongest diffuse light*; to this light the leaf-blades place themselves at right angles. Such a position insures that, on dull days at least or in shady situations, a maximum amount of light shall be absorbed, while direct sunlight never appears to injure these leaves, simply because it is constantly changing its direction during the course of the day. In certain plants, however, the so-called 'Compass plants' (Stahl, 1881), the fixed light position is determined by direct sunlight. These plants, among which the indigenous *Lactuca scariola* may be included, show the ordinary leaf orientation in shady situations, but in exposed places their leaves perform certain bendings and twistings so as to turn the laminae vertically and approximately north and south. The leaves thus stand in profile at midday, while in the morning and evening the surfaces are exposed to the incident rays. So far as the leaves are concerned which arise on the north or south aspects of the stem, a torsion at the base is sufficient to bring them into the profile position; those pointing east and west, on the other hand, have to carry out complicated movements in order to attain the vertical N–S position, for they do not rest content with merely bending upwards so as to press their upper surfaces against the stem, but exhibit curving on their midribs as well, northwards or southwards.

The leaves of the Compass plants in the mature condition are not dorsiventrally constructed, their east and west surfaces have the same structure. A bilateral construction and an accompanying vertical position of the leaf surfaces occur very frequently, e.g. in *Iris*, and in many New Holland species of *Acacia* and Myrtaceae. In the case of *Iris*, the vertical position of the leaf, although the orientation is not associated with definite points of the compass, is nevertheless an adaptation by which the brightest rays, at least in the height of summer and in southern lands, do not fall on the lamina so completely as would be the case were the blade horizontal; on the other hand, individual

leaves of *Acacia* and of the Myrtaceae, which are arranged so as to be vertical not to the soil but to the branch which bears them, are exposed at right angles to the full midday sun. It is doubtful, therefore, whether the orientation of the leaves is to be regarded in this case as a protection from excessive insolation. It is possible that these leaves are in general not affected at all by light. This is certainly true of many radially constructed leaves, such as those of the pine, *Sedum acre*, &c., whose fixed light position is such that the upper sides have as often the highest photic ration as the lower sides.

It is impossible for us to enter into the wider problems connected with the actual photic ration of leaves of different types, or with the relative photic ration of the upper as contrasted with the under side; we must content ourselves with quoting only one fact from the elaborate researches carried out by WIESNER (1899). This author showed that in addition to leaf types which avoid excessive light by their *orientation* (articulated leaves, Compass plants) there were also others that achieve the same results by their *form*. It may be experimentally proved that more light always falls on a flat leaf than on one which is concave or convex, and hence it may be assumed that the very common occurrence of leaves with uneven surfaces indicates an adaptation for the protection against excessive insolation.

It will be necessary for us to strictly limit ourselves in the selection of further illustrations of dorsiventral heliotropic organs. Omitting the consideration of flowers altogether (NOLL, 1885–7; SCHWENDENER, 1892) let us glance only at certain organs, which are not from the beginning dorsiventral but which become so as the result of the action of external factors. The seedling of the cucumber is orthotropic and positively heliotropic; but when it has reached a certain size a sharp curvature appears above the cotyledons, which is determined by the source of light, but which does not bring the epicotyl into a rest position in the direction of the light, but places it horizontally. The shoot continues to grow in this direction, becoming quite dorsiventral, for roots arise on its under surface (CZAPEK, 1898 b). We have a counterpart to the cucumber in *Hedera helix* (SACHS, 1879, p. 272). In this case the shoot is *negatively* heliotropic and grows in an approximately horizontal direction. Thus seedlings of *Hedera* and *Cucurbita*, planted side by side and exposed to unilateral illumination, bend their shoots in opposite directions, and we may watch the development of a perfectly plagiotropic shoot from an orthotropic, negatively or positively heliotropic plumule. Aerial roots also arise on the under side of the stem of *Hedera*, but this dorsiventrality is by no means inherent and fixed, for, as is well known, it is possible, by altering the direction of the light, to make any flank dorsal or ventral at will, and probably the same is true of *Cucurbita*. When rotated on a klinostat, both plants, if illuminated equally all round, are orthotropic. Markedly dorsiventral shoots are generally not so, if they be taken out of their rest position they do not attempt under all conditions to regain it by curving or torsion, but endeavour to adapt themselves to the new position by altering their structure.

Marchantia behaves quite differently. The gemma, from which we may cultivate the plant, is bilateral, and the side which is more strongly illuminated becomes the upper side of the thallus; the dorsiventrality, once established, cannot be altered by altering the illumination. If the direction of the light be changed, the plant behaves essentially like a foliage leaf, for FRANK has shown that not simple curvatures of the thallus only but torsions as well are set up which bring it back to the original light position (compare SACHS, 1879; CZAPEK, 1898 a). We may also refer here to the behaviour of certain lateral branches, whose initials are radial, but which, under the influence of unilateral light, become dorsiventral, just as they do when influenced by gravity (p. 453). The leaf insertions may remain unaltered, and only the petioles or the leaf bases induce a twisting of the *lamina* towards the flanks

(*Acer, Abies*), or photogenic torsions may be set up in the stem (*Cornus mas*, &c.), which serve the same purpose, i. e. of bringing the *point of insertion* of the leaf on to the flank.

Our summary of heliotropic phenomena has been brief and that for two reasons. It would be quite superfluous to treat of orthotropic organs in great detail, because in their case the phenomena are generally speaking perfectly obvious, but that is not the case with the plagiotropic organs. The phenomena exhibited by such organs are very complicated, and investigations in individual cases leave much to be desired. What we have said, however, may serve as an introduction to the more important general problems we have yet to study.

It was pointed out at the commencement of this lecture that the old explanation of heliotropic curvature was applicable only to orthotropic, positively heliotropic organs and that on that account it must be rejected. We must now investigate more closely what exactly takes place in heliotropic movements, so as to obtain if possible some insight into the mode of operation of the stimulus. Our knowledge of stimulus action has been greatly extended since DARWIN (1881) showed that, in certain cases, the heliotropic movement could make itself apparent at a considerable distance from the place which was unilaterally illuminated. Since then ROTHERT (1894) has repeated and extended DARWIN'S experiments, and nowadays this field of research is perhaps the most accurately studied in the whole range of plant physiology. With the view of obtaining some conception of the characteristic relations which exist let us observe the behaviour of seedlings of *Setaria* or some other member of the Paniceae group of the Gramineae. As in all members of that order, a primary sheathing leaf is formed above the scutellum, and this leaf we will, for the sake of simplicity, term the cotyledon. It is of a tapering form, and in a short time reaches its definite length of 3–6 mm., while the underlying, somewhat narrower region, the hypocotyl, grows much longer and may become 5–6 cm. in length. In seedlings of a certain age the cotyledon is full-grown although the hypocotyl still goes on growing vigorously. While, at first, growth in the hypocotyl is general through its entire length, later on the base ceases to exhibit any signs of increase, the most vigorous growth being confined to a region just below the apex. If such a seedling be illuminated on one side, a sharp heliotropic curving takes place at the apex of the hypocotyl, which creeps gradually backwards as far as growth will permit (Fig. 148). This curvature makes itself apparent only if the *cotyledon* be illuminated from one side whether the hypocotyl be exposed to light or not. If the cotyledon be shaded and the light be permitted to fall on one side of the hypocotyl no heliotropic curving takes place. Hence we may conclude that it is only the cotyledon that is sensitive to the light stimulus, and that it is only the hypocotyl which can carry out the movement. The excitation which the light effects in the cotyledon must be transmitted to the hypocotyl, and the curvature takes place only from such a transmitted excitation. We have thus in this case a definite organ for the perception of the stimulus of light, viz. the cotyledon, and, as ROTHERT has shown, it is more especially the apex of that organ that is the sensitive part; on the other hand the motile organ, the hypocotyl, is some distance away from the sensitive organ, and in it the power of perception is entirely absent. From the behaviour of these organs we may draw the further conclusion that perception and heliotropic excitation are two distinct phenomena—a point to which we have already drawn attention in speaking of geotropism (p. 444)—which depend on different properties of the protoplasm and which are independent of each other in so far

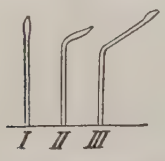


Fig. 148. Seedlings of *Setaria viridis*. *I*, straight etiolated seedling of median age. *II*, the same curved heliotropically. *III*, the same after being longer exposed to unilateral illumination. After ROTHERT, 1894. (About nat. size.)

that although an excitation always follows a perception (direct excitation) it does not follow that every excitation must be the *direct* consequence of a perception occurring in the region concerned, since excitations may also be transmitted (indirect excitation). We may therefore conclude from this experiment that these two types of excitation are fundamentally distinct processes, for it is only after indirect or transmitted and not after direct excitation that a reaction occurs in the case of the seedlings of the Paniceae. Since, however, we observe that a curvature follows in the *young* cotyledon also after a *direct* excitation, we may take it as *certain* that the phenomena of excitation in the cotyledon are in all cases identical with those in the hypocotyl, and that the non-appearance of curvature in the cotyledon in its later developmental stages is solely due to the cessation of growth.

The phenomena just described as occurring in young plants of *Setaria* are comparable to those which are seen in the majority of Gramineae (i.e. Poaeoideae). In them no hypocotyl is developed, and the cotyledon attains very considerable dimensions. It is sensitive to unilateral illumination throughout its *entire length*, but the excitation, and also the reaction, is most vigorous when the *apex* is subjected to unilateral illumination. This conclusion may be arrived at from a study of several facts. The course of ordinary heliotropic curvature supports this view. If we consider this phenomenon in *Avena* (Fig. 149), we note that that curvature begins just below the apex (*b*), but after 3½ hours (*c*), the whole organ, has become affected; later on the apical region (after several oscillations forwards and backwards, probably of an autotropic nature) again becomes straight and the curving is localized at the base, the radius of curvature being at the same time reduced (*d*). If we next attempt to determine the distribution of growth in the seedling, we find that the maximum is somewhere about 5 to 10 mm. from the apex, and that from this point upwards it becomes reduced very rapidly, but that, basally, the decrease is quite gradual. The heliotropic curving does *not*, therefore, begin in this case at the zone of greatest growth, but in a region where growth is very feeble, and hence it follows that the excitation must be greater at the apex than lower down, since, if the excitation were equally great throughout, the curvature must obviously start in the region of most vigorous growth. We may arrive at the same conclusion with regard to the distribution of heliotropic *sensitivity* by entirely different means. Heliotropic curvature appears in *Avena* if we illuminate the whole cotyledon on one side or only its base or only its apex, but the results are not identical in the three cases. The most remarkable result is obtained when we arrange, by means of a sheath of black paper of the form represented at Fig. 150, *I*, that the light falls on the upper end of the seedling from the right only, and on its base from the left. After 1½ hours the parts exposed to the light have bent in the form of a double bow, and the whole structure takes the form of an S. After five hours (Fig. 150, *II*, *III*) the excitation transferred from the apex downward has annulled the tendency of the base to bend to the left or even transformed it into a reverse tendency; in other words the transmitted excitation prevails over that locally developed. The apical zone, specially affected by the light, is very limited in extent, being at most 3 mm. long. From these experiments it follows that the *excitability* of the base of the seedling (as measured

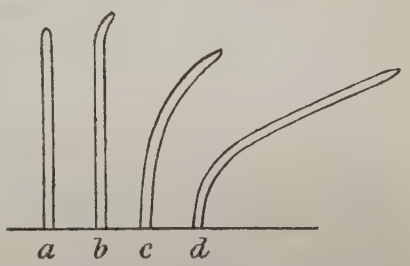


Fig. 149. Heliotropic curvature in *Avena*. After ROTHERT (from DETMER'S Practical Botany). 1½ nat. size. *a*, at the commencement of the experiment; *b*, after 1½ hours; *c*, after 3½ hours; *d*, after 9½ hours.

by its reaction) is not less than that of the apex, but its power of *perception* must be less well developed. When we speak of a more limited sensitivity, we mean the antecedent perception and not the excitation, still less the movement, but, generally speaking, the greater the sensitivity the greater the excitation, although movement, as we have seen, may fail to make itself evident in spite of a very great excitation.

From among the other examples which are known as supporting the hypothesis of a distinction between perception and reaction in heliotropic phenomena, or at least as confirming the idea of unequal excitation and the transmission of excitation to other regions, we will select the case of the leaves of the Malvaceae, whose behaviour has been carefully observed and described by VÖCHTING (1888). We have to deal with, in this case, a surface which, generally speaking, exhibits no active movements, and we have also an articulation immediately adjacent which may bring about curvatures by alterations in turgor, and finally a petiole showing growth movements. Necessities of space compel us to limit ourselves to the consideration of the movements of the articulation. Such movements may be induced in it directly, or they may result from an excitation transmitted to it from the lamina.

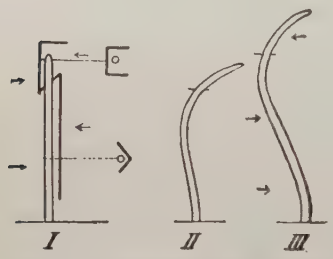


Fig. 150. *I*, Arrangement for partial darkening of a seedling of *Avena*. The arrows indicate the direction of the light. *II* and *III* show the result after 5 hours. The horizontal line indicates the limits of the regions subjected to the influence of the rays from opposite sides. *II*, the curvature to the right has been transmitted to the base. *III*, the seedling has curved to the right in its upper part, and to the left at its base. After ROTHERT (1894, pp. 17 and 59).

If unilateral illumination affects the pulvinus *only*, it behaves almost like a positively heliotropic stem, and when it bends it gives thereby a different inclination to the leaf-blade. The *leaf surface*, however, so influences the pulvinus that it places the blade at right angles to the incident rays. When VÖCHTING succeeded by appropriate experimental means in bringing the pulvinus and the blade into opposition, he found that the pulvinus, in obedience to the impulse transmitted to it from the lamina, responded much more readily than it did to a direct stimulus, and the direct stimulus was completely neutralized by a contrary stimulus transmitted to it from the blade. The perceptive power of the *blade* in other cases also is doubtless responsible for the movements of the petiole.

Further, proof has been adduced by KOHL (1894) in support of the view that the negative heliotropism of roots arises from a power of perception located in the root apex. As a result of our studies in the localization of heliotropic perception in the apices of grass cotyledons and of roots, we might be inclined to conclude that geotropic perception is also localized, but such a conclusion drawn from analogy must be rejected, first because geotropism and heliotropism are very different phenomena looked at from a physiological standpoint, and secondly because, from the biological point of view, heliotropic perception, in the apex of the cotyledon, in the apex of the root, and in the surface of the leaf-blade, appears to be entirely purposeful, while the object of localizing geotropic perception in the root apex is not apparent on the face of it.

Neither is it true that a definite organ is constructed in all cases to perceive the light stimulus, nor is the power of perception less intense at the region of the movement than elsewhere, for there are plant organs which exhibit as great powers of perception in the motile zone as in other regions, and yet in their case also the heliotropic excitation can be transmitted (ROTHERT, 1894).

Having now studied sufficiently in detail the behaviour of the grass seedling, and having, in the course of our study, seen that a heliotropic curvature must

consist of at least four distinct processes, i. e. perception, excitation, transmission of the excitation, and curving, let us examine these processes separately, as far as the present state of our knowledge will permit. Let us look at perception first. In the case of gravity, we could at least affirm with certainty that the first purely physical effect on the plant must be the influence of weight on the sensitive protoplasm, and we arrived at that conclusion because the effect of gravity could be replaced by centrifugal force. We cannot, however, so far replace sunlight by any other agent, and so we are compelled to seek some other solution of the problem. It may certainly be supposed that light operates in this case just as gravity does; in this case, as in that, we have to consider in the orthotropic organ a change in the direction of the active agent concerned, here as there we have to deal with phenomena due to unequal growth. SACHS laid special stress on the fact (compare MÜLLER-THURGAU, 1876, and SACHS, 1882) that the heliotropic rest position of an organ lies in the *direction of the incident rays*, just as the geotropic rest position lies in the direction in which gravity acts. A stimulus is administered whenever the long axis of the organ forms an angle with the line of operation of the stimulus. We do not know whether, from this correspondence between geotropism and heliotropism, SACHS himself drew the conclusion it suggests that gravity and light were comparable from a purely physical point of view; at all events it might be possible to conclude so and to advance the hypothesis that gravity like light depends on undulatory motions of ether; one might do so if indeed the likeness between geotropism and heliotropism, established by SACHS, were proved to be one of complete conformity of the two stimulation phenomena; but this is *by no means* the case.

It is possible, indeed it is highly probable, that the *reaction* or *curvature* is the same in both cases, at present, at least, we know of no difference. The heliotropic *excitation* may indeed be the same as the geotropic, but there is a great difference in the *perception* in the two cases, as is shown quite clearly by certain observations made by CORRENS (1892). When CORRENS studied the effect of oxygen on tropistic movements, he was able to establish the fact that the seedlings of *Helianthus* in the presence of the slightest traces of oxygen, were still capable of exhibiting geotropic curvatures, while a far higher percentage was needed (1 per cent. of the normal amount) for the performance of heliotropic movements. Geotropic curvature ceased when growth came to an end, hence it is possible that in this case perception was quite independent of the presence of oxygen, while heliotropic perception appeared only when relatively large amounts of oxygen were present.

Although it follows of necessity from this fact that geotropic and heliotropic perception are essentially different, still SACHS's view might yet be correct, according to which it is the *direction* of the incident ray that is perceived. In order to estimate the truth of this theory more accurately we must compare it with the earlier conceptions of heliotropism. According to these the essential condition for heliotropic curving is an unequal *intensity* of light on either side of an orthotropic organ. The rest position would thus be in a line *parallel* with the path of the light, because when so placed all sides of the organ would be equally brightly illuminated; when unilaterally illuminated, the fact that the light rays stream through the plant in an oblique direction, would not lead to perception, but that result would be attained by the unequal illumination of the two sides.

If we compare this conception with SACHS's views, we are driven to the conclusion that it is impossible to accept the explanation previously offered by DE CANDOLLE, that the result is due to the *direct action* of light of different intensity on the rate of growth. We have already (p. 461) refuted this theory, and the behaviour of the Gramineae, studied above, is sufficient in itself to disprove it.

We may rather inquire as to the origin of the stimulus which brings about the perception, and here we meet with two alternatives, 'Does the plant perceive the *direction* of the light rays or the *difference* between the *illumination* on either side of its body?' As a matter of fact the side of the plant turned towards the source of light must be more brightly illuminated than the side turned away from it, and if the long axis of the plant be parallel with the light ray all sides will be equally illuminated. For simplicity let us limit ourselves to orthotropic organs only. SACHS's hypothesis has never been *proved*, and the facts which are recorded in MÜLLER-THURGAU's memoir (1876), and on which, according to SACHS, these views are founded, appear to us to support equally well the other view. The heliotropic curvature exhibited by unicellular and very translucent plants or plant organs (such as Fungi or roothairs) appears at first sight most in harmony with the facts on which SACHS's theory is based, for in these cases it may be affirmed that there is practically no difference in the light intensity on the concave and convex sides, seeing that the amount of light lost by absorption in the cell is not worth considering. The difference has not, however, been measured, nor do we know how great it must be in order to lead to perception in the plant.

More recently SACHS's views have been vigorously attacked by OLTMANNS (1892). This author placed the plants on which his experiments were made in a box into which light entered from one side only, the light being direct sunlight. The light was made to pass first of all through a hollow glass prism filled with gelatine tinted with indian ink. When the apparatus was arranged as is shown at Fig. 151, the sunlight struck the prism at right angles, and hence the light rays passed (in the direction of the arrows) into the space below, parallel to each other, while the intensity of the light obviously diminished from left to right. Behind the prism straight-growing filaments of *Vaucheria* were placed, and it was found after several hours that those which were subjected to light of medium intensity had remained quite straight, whilst those to right and left had curved apically towards them. The curvatures took place in a plane *parallel* to the outer wall of the box, *at right angles* to the direction of the rays, positive or negative according to the intensity of the light. Unfortunately the distribution of the light intensity and the course of the rays were as simple as that described only in a few of OLTMANNS' experiments, and even in this one it is still a matter of doubt whether the rays do not also travel in the same directions as those which the curvatures followed. Although, doubtless, a decrease in the intensity of light may *theoretically* be obtained independently of the direction of the rays, in practice, in the most carefully planned experiment, owing to the reflection of the light from the walls of the vessel, the dust particles, and from the plant itself, deviations must occur.

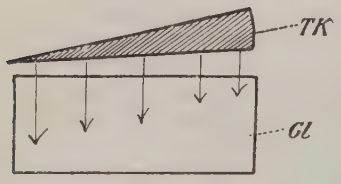


Fig. 151. OLTMANNS' apparatus. *Gl*, glass vessel containing the plants to be experimented on; *TK*, prism. The direction of the light and its intensity are indicated by the arrows.

DARWIN (1881) also carried out a research, which aimed at proving that heliotropic curvature does not take place in the direction of the rays. In order to carry out this experiment most appropriately a seedling of *Setaria* is illuminated on two sides, which we shall term 'right' and 'left', by parallel beams of light of equal intensity, under which conditions naturally the seedling remains uncurved. If the whole of the *posterior* half of the photosensitive cotyledon be darkened by means of a tinfoil cap while light is permitted to penetrate the anterior half as before, one would expect, if SACHS's view were correct, that no heliotropic curvature would take place. As a result of DARWIN's

experiment, however, there can be no doubt that heliotropic curvature does take place and that too, forward, i. e. in a plane at right angles to the incident ray. According to the hypothesis which regards the inequality of illumination on opposite flanks as the origin of the stimulus in heliotropism, this result is quite intelligible, but we have yet to inquire whether this result really contradicts SACHS's hypothesis. Apparently it does not, since it is possible to imagine that, owing to refraction and reflection in the *interior* of the cotyledon, the rays may be deflected in a direction at right angles to the course followed outside.

It is perhaps impossible to arrive at a decision as to which of the two hypotheses is the correct one, because it is scarcely possible to obtain a definite direction of light rays without inducing differences in intensity at the same time, and because differences in intensity cannot be attained without light diffusing from a brighter to a less bright region. It is a matter for individual judgement which hypothesis is to be accepted; on the ground of *analogy* (Lecture XXXVII) we lean to the hypothesis based on *difference of intensity of light*. If that be so, we must assume that the plant has the power of *comparing* the degree of illumination on different regions. Orthotropic organs perform heliotropic curvatures when opposite flanks are unequally illuminated, which curvatures have the effect of cancelling this difference. Plagiotropic organs, on the other hand, are attuned to unequal illumination. Looking at orthotropic organs only, it is obvious that the liminal stimulus may be exceeded when a *certain* difference is reached, but this can only be determined by *experiment*. Such experiments have been carried out by MASSART (1888). He exposed *Phycomyces* in appropriate ways for two hours to unequal illumination on opposite sides, and found that a positive heliotropic reaction took place when the relationship between the two light intensities was at least in the ratio of 100 to 118. This ratio was found to be constant for light of varying intensity. Thus MASSART was enabled to prove in the case of heliotropism the validity of WEBER'S law as to the relation subsisting between the amount of the stimulus and sensitivity, a law which we shall have to refer to later on in reference to other stimulus phenomena, and thus he was able to confirm an earlier suggestion made by PFEFFER (1884). Further investigations are urgently needed, however, since the results we have already arrived at (p. 463) as to this power of adjustment show most clearly that the law can apply only to light of *certain* intensity. With light of higher intensity *no reaction* occurs (in the indifferent condition), and obviously also when the degree of illumination on opposite sides reaches higher relative proportions than 100 to 118. In opposition to MASSART'S results, we must expect also the difference in illumination leading to a stimulus movement to vary with the absolute light intensity, but the laws governing this change have yet to be discovered. The answers to very many questions, which cannot even be indicated here, will depend on such experimental researches.

Experiments are certainly not wanting in modern literature which have for their object the determination of the liminal intensity in heliotropism (compare p. 462; FIGDOR, 1893). They all deal, however, with unilateral light alone and only one special case is considered, viz. how weak the light may be which still induces heliotropic curvature, if the opposite side be shaded as much as possible.

The latent period of the heliotropic stimulus has already been determined. According to CZAPEK (1898 a) it amounts to 7 minutes in the cotyledons of *Avena* and in *Phycomyces*; 10 minutes in the hypocotyls of *Sinapis alba* and *Beta vulgaris*, 20 minutes in the hypocotyl of *Helianthus* and 50 minutes in the epicotyl of *Phaseolus*. If one of these organs be unilaterally illuminated for the specified time, heliotropic curvature ensues afterwards in the dark, that is to say, we meet with an after-effect in this case as in geotropism. We are quite ignorant,

however, as to whether and how far the latent period is dependent on the *intensity* of the light.

While in the case of gravity in nature we have to consider only one variable, i. e. the *direction* in which the force acts, in the case of light as a stimulus we have several variables, viz. the *direction*, the *intensity*, and yet another, which we have not as yet considered, viz. the *quality* of the light. It has long been recognized that rays of different wave length do not act in the same way. The more highly refrangible rays which are more especially concerned in the formative activity of light (p. 310) have been found to be also those especially concerned in heliotropism. WIESNER (1878) found that the rays at the limits of the violet and ultra-violet regions were the most active, and that the activity decreased from that point so that, in yellow light, practically no heliotropic curvature took place at all. [Compare DANDENO, 1903.] The movements begin again, however, in red light and increase towards the ultra-red, although this is not true of every plant examined. Negatively heliotropic organs, according to WIESNER, behave like positively heliotropic ones.

Examining more closely the action of light, we have to recognize in the first instance in all cases a purely physical or chemical action which may lead to a heliotropic stimulus if the light falls unequally on different sides; wherein the primary effect lies, however, we are quite ignorant. Possibly light may first of all induce certain chemical changes; but an action like that which takes place in silver salts is inconceivable, because the red rays, which perform no function in photography, were found to be active in WIESNER'S experiments. It is certainly possible that heliotropic curvature in red rays is a phenomenon *sui generis*, which may be compared with thermotropic processes, which we shall consider later on (Lecture XXXVII), and if that be so, heliotropism might be, in the restricted sense, included amongst those light effects which, for the sake of brevity, may be termed photographic. On the other hand it is also conceivable that definite chemical reactions are rendered possible owing to the action of those kinds of rays which WIESNER found to act heliotropically.

Meanwhile we need not consider further the so-called chemical effect of light. If it can be proved that the plant reacts, as we may say, not to *light* but to an *effect* produced by light, then heliotropism may be regarded as a special instance of chemotropism (Lecture XXXVII) and would, for that reason, be still further removed from the category of phenomena that geotropism belongs to, where, as we have seen, it is not gravity itself as such, but the actual weight associated with gravity that is perceived. Still the difference between geotropism and heliotropism is sufficiently great to cause us to treat these two phenomena separately; the likeness lies in the nature of the reaction, in the character of the curvature; the differences lie in the nature of the perception.

In addition to the chemical effect of light we have also to consider a *mechanical* influence. According to MAXWELL, a *pressure* amounting to about 0·5 mg. per sqm. is induced in any medium through which a light wave is propagated, in the direction of the path of propagation. The existence of this pressure has been more recently confirmed experimentally by LEBEDEW. Apart altogether from its limited amount there are other reasons for doubting whether heliotropic perception is at all dependent on this pressure. [HABERLANDT (1905) has recently advanced a unique hypothesis as to light perception in plants. On the upper epidermis of many plants papillae are to be found which, according to HABERLANDT, act as converging lenses. The papilla concentrates a spot of light on the protoplasm lining the inner wall of the epidermal cell. The protoplasm of the inner wall is sensitive to this light and is able so to orientate itself in response, that, according to the position of the leaf in regard to the source of light, the light is concentrated at different places on the inner wall

of the cell. As yet the essential basis, however, for this very ingenious theory is still wanting. It is remarkable that these 'ocular' papillae are not present on orthotropic organs nor on the highly sensitive seedlings of the Gramineae. Further there is no evidence forthcoming in support of the view that the upper side of the leaf only is capable of appreciating the light stimulus. The experimental evidence in favour of the function of the ocular cells will scarcely stand critical investigation.]

We have now to inquire into the other processes which follow on perception of the stimulus. We have already referred to the excitation, the transmission of the stimulus, and finally the reaction. Meanwhile there is still a further phenomenon which must be noted; we saw that the plant must be able to *compare* the different intensities of light on different sides, so that we are now met with the question as to whether the plant compares the perception itself or the excitation. It is possible to hold the view that it does neither, but that the case is of a more complicated nature. Probably the light operates on *each* individual cell as a stimulus and induces in each a reaction, at present unknown to us. If these individual reactions be uniformly alike, then no further effect is produced, but if they be dissimilar, this difference induces an heliotropic excitation. As to the nature of excitation we know nothing, and as to its transmission also very little is known. In ROTHERT's (1894) experiments with grass seedlings, it was possible to determine only that the excitation travelled towards the base, following the path of the parenchyma. We are compelled to assume that the intercellular protoplasmic threads are the immediate agents in the transference of the excitation from cell to cell, but such a fibrillar structure as that described by NĚMEC (1901) cannot play any special part in the transmission (HABERLANDT, 1902). Finally, as to the last link in the chain, viz. the reaction, the immediate cause we are at least acquainted with, and that is either differential growth or differential turgor-stretching.

We see, therefore, that our knowledge of the very important problems which heliotropism presents to us is as yet very meagre; many of these questions lend themselves to experimental treatment and possible solution, and these may in turn throw unexpected light on the remainder.

Bibliography to Lecture XXXVI.

BERTHOLD. 1882. Jahrb. f. wiss. Bot. 13, 569.
DE CANDOLLE. 1832. Physiologie végétale, 3, 1086.
CORRENS. 1892. Flora, 75, 87.
CZAPEK. 1898 a. Jahrb. f. wiss. Bot. 32, 175.
[CZAPEK. 1898 b. Flora, 86, 425.]
[DANDENO. 1903. Science, 18, 604.]
DARWIN. 1881. Bewegungsvermögen. German ed. by CARUS. Stuttgart.
FIGDOR. 1893. Sitzungsber. Wiener Akad. Math.-nat. Kl. 102, I. Abt. 45.
FRANK. 1870. Die natürl. wagerechte Richtung von Pflanzenteilen. Leipzig.
HABERLANDT. 1901. Biolog. Centrbl. 21, 369.
[HABERLANDT. 1905. Die Lichtsinnesorgane d. Laubblätter. Leipzig. See also Ber. d. bot. Gesell. 22, 105.]
HANSGIRG. 1890. Ber. d. bot. Gesell. 8, 353.
HOFMEISTER. 1867. Die Lehre von der Pflanzenzelle, p. 293.
KOHL. 1894. Die Mechanik d. Reizkrümmungen. Marburg.
LEBEDEW. 1901. Annalen d. Physik, IV, 6, 433.
MASSART. 1888. Bull. Acad. Bruxelles, III, 16, 590.
MÜLLER-THURGAU. 1876. Flora, 59, 65.
NĚMEC. 1901. Reizleitung und reizleitende Strukturen. Jena.
NOLL. 1885–7. Arb. bot. Instit. Würzburg, 3, 189 and 315.
OLTMANNS. 1892. Flora, 75, 183.

OLTMANNS. 1897. Flora, 83, 1.
PFEFFER. 1884. Unters. a. d. bot. Institut Tübingen, 1, 407.
ROTHERT. 1894. Cohn's Beitr. z. Biol. 7, 1.
SACHS. 1879. Arb. bot. Inst. Würzburg, 2, 226.
SACHS. 1882. Vorlesungen über Pflanzenphysiologie. Leipzig.
SCHWENDENER and KRABBE. 1892. Abh. Berliner Akademie.
STAHL. 1880. Bot. Ztg. 38, 412.
STAHL. 1881. Ueber sog. Kompasspflanzen. Jena.
VÖCHTING. 1888. Bot. Ztg. 46, 501.
WIESNER. 1878–80. Die heliotr. Ersch. i. Pflanzenreich (Denkschriften d. K.K. Akad. Wien, 39 and 43).
WIESNER. 1899. Biol. Centrbl. 19, 1.
WIESNER. 1901. Ibid. 21, 801.

LECTURE XXXVII

COMBINED ACTION OF GEOTROPISM AND HELIOTROPISM. THERMOTROPISM AND OTHER TROPISMS

HAVING now discussed separately heliotropic and geotropic movements, we have still to glance at the movements which result from the simultaneous or rapidly consecutive stimuli of light and gravity. We will confine ourselves to a discussion of the data which CZAPEK's (1895) recent researches have given us access to.

CZAPEK experimented with plants which, like the seedlings of *Avena* and *Lepidium*, exhibited a heliotropic curvature under optimum conditions in time and degree, like that of geotropism. He convinced himself that the plants mentioned when placed on the klinostat began to exhibit a curvature, when they were subjected to unilateral illumination, simultaneously with other seedlings placed horizontally and kept in the dark; further that the heliotropic curvature progressed in the same manner as the geotropic, and that the maximum amount of curvature (90°) was reached in both cases at the same time. The influence of *consecutive* stimuli was next investigated. The seedlings were kept horizontally in the dark until the first trace of geotropic curvature became apparent, when (about an hour after the beginning of the experiment) they were placed vertically and illuminated from one side, so that the ensuing heliotropic curvature might operate antagonistically to the geotropic curvature. Under these conditions a *reduction* in the geotropic curvature took place exactly at the same time that control plants which had not previously been subjected to geotropic stimulus began to show heliotropic curvature. This experiment, according to CZAPEK, teaches us that a primary geotropic induction has no effect on a subsequent heliotropic stimulation.

The case was entirely different if the seedling was *first of all* heliotropically stimulated and then laid horizontally in the dark in such a way that the side which was more brightly illuminated faced downwards. There was a very marked delay in the initiation of the geotropic reaction as compared with control plants which had not been heliotropically stimulated, and this delay was all the greater the longer the heliotropic stimulus was allowed to influence the plant; after a stimulus of ten minutes' duration it amounted to a quarter of an hour, after sixty minutes' stimulation the delay amounted to two hours. It would appear, therefore, that the geotropic reaction is affected by heliotropism, although as far as we are concerned the matter must not be taken as proved. CZAPEK's supposition that the two stimulus reactions, in the case of a solitary induction, take place in exactly the same time has yet to be more definitely

established. In another memoir CZAPEK (1898) has shown that, in the case of *Avena* and *Lepidium*, the latent geotropic period is twice as long as the latent heliotropic period, the former being 15′ the latter 7′. It may therefore be affirmed that, under given conditions, these plants are less sensitive to geotropic than to heliotropic stimuli. There is another important point which comes out on a critical examination of these results, viz., 'the moment of the commencement of the negative geotropic reaction in seedlings which had been curved backwards heliotropically was naturally considered as that at which the angle with the horizontal began to decrease.' Geotropic curvature, in other words, was *observed* when it had *overcome* the heliotropic after-effect. Obviously it would have been more scientifically correct to compare the experimental plants with controls which had been heliotropically stimulated for the same length of time and then rotated in the dark on a klinostat. The moment at which the curvature of the seedlings which had been geotropically stimulated took place after the initiation of the same movement in the plants on the klinostat would then have been the moment of the initiation of the geotropic movement. It is very probable that no delay in the initiation of the geotropic curvature would then have been noticed.

We believe that only one conclusion can be drawn from these experiments, a conclusion already suggested by the difference in the latent periods, viz. that in *Avena* and *Lepidium*, of two stimuli of equal duration, one geotropic and the other heliotropic, the latter has the greater effect. Hence a geotropic reaction in full swing is soon overcome by a heliotropic, whilst geotropism exerts only a gradual influence on heliotropic activity. CZAPEK'S assertion that subsequent heliotropism influences geotropism appears to us to be entirely without foundation. It must also be noted that CZAPEK himself did not observe it to occur universally. In plants like *Helianthus*, which responds more rapidly to geotropic than to heliotropic stimuli, no retardation of the geotropic reaction in consequence of a previous unilateral illumination was noticeable. In *Helianthus* geotropic curvature began at the same time as in *Avena*, only it was much more vigorous and hence earlier observable.

The behaviour of plants subjected to the antagonistic but *simultaneous* influence of light and gravity is of great interest. CZAPEK has carried out many experiments on this subject also, in continuation of those previously instituted by MOHL (1851), MÜLLER-THURGAU (1876), VÖCHTING (1888 b), NOLL (1892), and others. As a detailed discussion of all these researches would carry us too far, we will limit ourselves to a summary only.

1. If unilateral light falls on normally orientated plants, many of them place themselves directly in the line of the light rays (*Phycomyces*, *Pilobolus*, *Vicia sativa*), others (*Lepidium*, *Avena*) place themselves at a small angle with the path of the incident ray, others again (*Phaseolus*, *Helianthus*) bend but slightly from the vertical. The beginning of the heliotropic curving follows in all cases at the same time as it does in plants placed on the klinostat, but the heliotropic rest position is reached in general at a much later period.

2. If horizontally directed light falls on a plant already lying horizontally, the final result is almost the same as in 1. *Phycomyces* and *Pilobolus* continue their horizontal growth, *Avena* seedlings and those of many other plants form an angle of less than 20° with the horizontal, *Helianthus* and *Ricinus* find their rest positions at an angle of under 45°, and *Cucurbita* at an angle under 50°.

3. If light be projected from below in a vertical direction on the plant lying horizontally, *Avena* and *Phycomyces* bend at right angles downwards; others remain horizontal, others again (*Helianthus*) curve *upwards* at an angle of under 45°.

4. If, finally, light from beneath be projected on an *inverted* plant, e.g., *Helianthus*, it takes up a rest position at an angle of 45° *downwards*, while other plants keep on growing vertically downwards.

It is obvious, therefore, that in certain plants, such as *Avena*, heliotropism is always the dominant factor, whilst in other cases it is always geotropism that is predominant and such plants are less sensitive to heliotropism. CZAPEK has shown that, after *Avena* has taken up its rest position after stimulation by horizontal illumination, *first of all* an obvious geotropic upward curvature follows. Further he has found the same initial geotropic curvature occurring in horizontally laid plants which have been illuminated vertically from below, and this indeed occurs at the normal time and delays the heliotropic downward curvature. In this case one cannot assume that the geotropic curvature is finally overcome by heliotropism, and we must agree with CZAPEK in believing that the geotropic *perception* cannot be obliterated. The antagonism expresses itself in the reaction, although we are still entirely unable to show where it makes itself apparent in particular. Again it is quite possible that in other cases also the perception may be destroyed.

The observations which have been recorded on the combined action of geotropism and heliotropism do not as yet lead to any satisfactory general conclusions; they require revision and correction in many respects, because possibly the individual variants in CZAPEK's experiments have not been sufficiently excluded. The chief difficulty in all such experiments lies in obtaining equally great excitations by different stimuli. The goal aimed at is perhaps quite unrealizable, if *different and non-comparable excitations* correspond to *different stimuli*.

If the problem as to the combined influence of geo- and helio-tropism in orthotropic organs presents great difficulties these are greatly increased when we turn to plagiotropic, and more especially dorsiventral organs; so much so, indeed, that we will not attempt to enter on a discussion of the phenomena presented by them.

We have by no means exhausted the movements leading to orientation of plant organs, for there are still quite a number of stimuli which lead to movements of this character. That most closely related to light is *heat*, which also induces a special kind of movement. Since WIESNER (1878) established the occurrence of heliotropic curvatures due to the action of red and ultra-red light, so we may with equal justice speak of *thermotropic* curvature, for rays which pass through a solution of iodine in bisulphide of carbon are known as dark heat rays. Positive curvatures induced by such rays have been observed by WIESNER in *Vicia sativa* and in the cress. WORTMANN (1883), in a lengthy series of experiments, endeavoured to prove thermotropic curvatures in the sporangiophores of *Phycomyces* and in seedlings of *Lepidium*, *Linum*, *Zea*, &c. He employed as a source of heat a large vertically placed metal plate, which had been warmed and which gave off the heat rays from a darkened surface facing the plant; but in repeating WORTMANN'S experiments STEYER (1901) showed that WORTMANN'S apparatus was not a suitable one. This latter investigator by careful experimental means showed that in the case of *Phycomyces* no sign of thermotropism was exhibited, and that a seedling of *Lepidium* was positively thermotropic at high temperatures and negatively so at low. STEYER'S statements as to seedlings are also, however, wanting in detail, without which the subject cannot be considered as presented in a clearly intelligible form. At the same time we know for certain that *shoots* are positively thermotropic, since VÖCHTING (1890) has proved that the peduncle of *Anemone stellata* follows the course of the sun only on account of its positive thermotropism. That heliotropism has nothing to do with this reaction is shown by the fact that these movements go on in darkened chambers where the peduncle bends towards that part of the wall of the vessel which is most

heated by the sun at the moment. *Anemone nemorosa* and *Tulipa silvestris* behave in the same manner.

More exact investigations have been carried out by WORTMANN (1885) on *roots*. When roots are grown in water or moist air and heated on one side *no* curvatures take place, but when they are grown in sawdust thermotropic curvature was very evident. The response was the result of heat *conduction* not of *radiation*. WORTMANN placed the sawdust in zinc boxes 6 cm. broad, one longitudinal wall of which was heated to about 40° C. by being brought into close proximity to a gas jet, while the other was kept at a temperature of about 9° C. by means of running water. The temperature of the sawdust on either side thus showed a difference of about 30°, roughly 5° C. for every cm. of diameter. Every root grown in this medium was thus necessarily warmer on one side than on the other; further, the roots collectively were also, according to their position, subjected to higher or lower temperatures. The thermotropic curvatures resulting were markedly different, those exposed to higher temperatures showing negative curvature, those exposed to lower temperatures positive. This result reminds us of OLTMANNS' experiments on *Phycomyces* where heliotropic curvature was positive or negative according to light intensity. Just as there a state of indifference was brought about by light of medium intensity, so also we may anticipate a similar condition to arise in the root when it is exposed to medium temperatures. As a matter of fact, WORTMANN found that *Ervum lens* exhibited negative curvature only between 27·5° C. and 50° C., and positive only between 26° C. and 12° C. At 27° C., the critical temperature, the reaction was sometimes positive, sometimes negative, and sometimes there was no reaction whatever. In the case of *Pisum* the critical temperature was about 32°, in *Zea mais* rather higher, i. e., about 38° C. In other cases (e. g. *Phaseolus*) negative curvatures only could be induced.

Additional investigations on roots have been carried out by J. AF KLERCKER (1891), who measured the angle made by the curved root with the vertical after the completion of the reaction. The following results on an average of 28 experiments were obtained from *Pisum*:—

Temperature	26°–29°	29°–32°	32°–35°	35°–38°	38°–41°
Inclination	8·9°	12·9°	27·2°	38·4°	43·9°

In these experiments we are dealing only with negative curvatures, and we see at once that the stimulus increases markedly with the temperature; in like manner we observe, in the case of positive curvatures, an increase of the stimulus as we recede from the critical temperature. This is very well shown by *Sinapis alba*, where, at 24°–29° C., the angle is only 2·4°, but amounts to 19° when the temperature is 19°–24° C. On the analogy of the heliotropic curve (p. 463) it is very probable that when the temperature falls still further the stimulus will again decrease; e. g., KLERCKER found that in *Sinapis*, when the temperature was 14°–19° C., the angle was only 10·5°. It is very desirable that a complete curve should be obtained for one and the same organ, from the highest to the lowest temperatures, for it is obviously only in this way that the fundamental facts with reference to thermotropism can be fully established.

If, in spite of the imperfect nature of our knowledge, we inquire into the cause of the stimulus in thermotropism, we meet with the same differences of opinion as in the case of heliotropism. VAN TIEGHEM (1884), who was the first to draw special attention to thermotropism, put forward a theory, corresponding in all respects to that advanced by DE CANDOLLE for heliotropism. He argued from the known facts as to the influence of heat on longitudinal growth (p. 300). When an organ is subjected to a temperature lower than the optimum, the warmer side elongates more vigorously than the other, and the curvature is consequently negative in character; if, however, the temperature be above the optimum, the cooler side will grow

more vigorously and a positive curvature towards the source of heat will ensue. As we may readily understand, the response in the case of the *root* does *not at all* correspond to that of the stem, and hence WORTMANN (1885) was perfectly right in repudiating VAN TIEGHEM'S hypothesis. In *some* cases, certainly, this theory may explain the facts. Thus VÖCHTING (1888 a) carried out a careful investigation into the mode of unfolding of the buds of *Magnolia*, where the curvatures are negative owing to the illuminated side growing more rapidly than the shaded. That this is due to heat alone and not to light, has been conclusively proved by VÖCHTING. In all probability, however, this is *not* a case of thermotropic movement, for in other organs, e.g. fruits, similar movements may be induced, if they be made to grow more rapidly on one side than the other. Heat does not act in this case as a specific stimulus, but as a 'formal condition'. Generally, however, this is not the case, for WORTMANN (1885), in some experiments, found that those parts of the roots grew most vigorously which were subjected to a temperature, which, operating on all sides of the organ, *did not permit of any further growth* (supra-maximum temperature).

After having refuted VAN TIEGHEM'S hypothesis, WORTMANN assumed that in thermotropism we are dealing with the *direction* in which the heat rays penetrate the plant. He has advanced no proof of this, however, and it could only occur in cases where *radiant* heat was the cause of the thermotropism. As far as regards roots grown in sawdust, we cannot speak of heat *radiating in a definite direction* since diffusion of heat by conduction is the first consequent; *there is indeed a direction of temperature decrease, but not of heat rays*. So far as we know, however (compare VÖCHTING, 1888 a), thermotropism due to *radiant* heat cannot be distinguished from thermotropism due to *conduction*. We are entitled to assume that the cause of the stimulus is the same in both cases, and that it lies in the *dissimilar temperature on opposite sides*, a difference which the plant recognizes and to which it reacts. Obviously, growth may express itself on the individual sides quite differently from what it does when all sides are subjected to a uniform and equally high temperature. In heliotropic curvature also we saw that the illuminated side under certain conditions grew more rapidly than it did when all sides were equally illuminated. We are ignorant how great the difference in the temperature affecting the two sides must be before a stimulus is effected, nor has the effect of the height of the absolute temperature on the liminal intensity of the stimulus been determined, nor how the stimulus is increased by rise of temperature. In this direction there is ample room for experimental inquiry.

As to the purely physical or chemical aspect of heat as a stimulus, looked at from the point of view of perception, we know nothing. We have compared thermotropism with heliotropism, but at the same time we do not mean to imply that the perception which precedes curvature is the same in both cases. That goes without saying, for one of the data which WORTMANN has established points indeed quite the opposite way; roots which have their apices removed exhibit thermotropic curvature, and hence the root apex cannot be the organ of perception, or at least cannot be so exclusively, although we must admit it to be so in the case of heliotropism.

Associated with heat is *electricity*, which is propagated in waves by radiation or conduction. Although it has recently been shown that radiant light, heat, and electricity are closely related forms of energy, which differ from each other only in wave length, we must not conclude that on that account they operate on the plant in the same way. The variation in the *amplitude* of the waves, which in the case of light are infinitely small in relation to the plant, but which in the case of electricity markedly exceed the diameter of a seedling or a *Phycomyces*, may induce markedly different results in organisms. It is impossible to draw any conclusions in this connexion from the solitary experimental investigations conducted by HEGLER (1891), in which he established negative

electrotropism in *Phycomyces* ; hence it is preferable to await the accumulation of further data.

More comprehensive investigations are forthcoming as to the influence of electric currents than as to that of electric radiations. ELFVING was the first (1882) to observe curvatures in the root when galvanic currents were sent through the water in which the roots were bathed. These curvatures were sometimes positive, the root apex curving towards the positive pole, sometimes they were negative. According to ELFVING'S statements the direction of curvature depends in the first instance on the nature of the plant, according to BRUNCHORST (1884) it depends on the strength of the current. A strong current induces positive, a weak current negative curvature, while medium currents produce no effect at all. The medium current is not the same, however, for every plant. At first sight the results obtained in this relation appear to exhibit a certain likeness to the variation in the heliotropic response induced by different intensities of light. The likeness is, however, superficial since, as BRUNCHORST has shown, the positive galvanotropic curvatures are generally not phenomena of stimulation, but originate entirely from the fact that growth on the positive side of the root is injured by the current. Probably certain chemical changes are induced by the current which operate first of all inhibitively to growth and finally fatally ; at all events a root which exhibits positive curvature of this character, always dies, not only on that side but altogether, at the latest after 24 hours. The negative curvatures on the other hand appear to be genuine phenomena of stimulation, where the root apex acts as the organ of perception. We have even less ground for assuming that the plant has the power of appreciating electric waves themselves than we had for believing in its power of perceiving light and heat waves ; in all probability the action of the current is to develop certain bodies by electrolysis, which lead to appreciation of the stimulus (compare Lecture XLIII).

The unequal distribution of certain soluble substances may also bring about directive movements. These have been termed *chemotropic* movements and are especially well seen in Fungi and in pollen-tubes. MIYOSHI (1894 a) has confirmed this in the case of certain Mucorinae and in *Penicillium*, *Aspergillus*, and *Saprolegnia*, the occurrence of movements in which was recognized long before and which can be interpreted only as cases of chemotropism. Thus KIHLMANN (1883) found that the cells of *Isaria* became bent out of their previous path of growth when placed in the neighbourhood of germinating spores of *Melanospora parasitica* and ultimately grew over them, and DE BARY (1884, 393) has suggested that the entry of parasitic Fungi into their host-plants is due to stimuli of a chemical nature. From MIYOSHI'S investigations there can be no doubt that chemotropic movements are widely distributed amongst Fungi and generally speaking serve the purpose of guiding the fungus to a suitable nutritive substratum, although chemotropic attraction is also effected by substances which are not good nutrients. MIYOSHI advanced further proof of chemotropism by injecting leaves, such as those of *Tradescantia*, with certain experimental solutions, and sowing spores of a fungus on the moistened epidermis. The injection diffused out through the stomata, and, if it was positively chemotropic, he found that the hyphae curved into the stomata, while they grew beyond them when the leaf was injected with water only. MIYOSHI obtained similar results by sowing the spores on finely perforated plates of mica smeared over with a chemotropic layer of gelatine. Finally he employed fine capillary tubes filled with the solution, the ends of which he inserted into the drop of fluid of an ordinary slide culture, thus permitting of a diffusion of the stimulating agent. Whenever a straight fungus hypha encountered dissimilar concentrations of the stimulant on different sides, its growing apex bent round until the new growth had placed itself parallel with the diffusion flow of greater concentration, towards which it grew. WORTMANN'S experiments on the thermotropic curvatures of roots (p. 479) present us with quite similar phenomena, for we may well com-

pare the temperature income with the diffusion inflow in this case. No one, however, would desire to defend the view that the direction of the diffusion inflow determines the orientation of the long axis of the cell; it is much more probable that the plant reacts to unequal distribution of the stimulant, and endeavours to place itself in such a position that all sides are in contact with equal concentrations of the stimulant. This, however, cannot be directly proved, because it is not possible to bring about stimulation on different sides by different concentrations, without producing at the same time a diffusion current.

Miyoshi investigated a large number of substances in different states of concentration, and was able to prove that some were good attractive media, others only moderately so, whilst others still, if stimulants at all, were repulsive. Repulsions, such as those exhibited by the cells of Fungi, have been observed after treatment both with free organic and inorganic acids; they were also induced by alkalis, alcohol, certain salts, such as potassium nitrate, magnesium sulphate, potassium and sodium tartrate, all in solutions of weak concentration. That the successful media did not act equally well on all Fungi examined is easily intelligible. The Hyphomycetes behave very like each other, but *Saprolegnia*, living as it does under very different conditions, reacts very differently. Exact investigations on the behaviour of such Fungi as are extreme specialists so far as their nutrition is concerned, are calculated to teach us much with regard to the special phenomena of chemotropism. Generally speaking, ammonium compounds, phosphates, peptone, asparagin, and sugar are good attractive agents; ammonium phosphate among inorganic compounds is especially so. The different types of sugar, prominently grape sugar and cane sugar, are excellent attractives for Hyphomycetes, but *Saprolegnia* responds but feebly to them. Glycerine and gum arabic act neither attractively nor repulsively, which indicates that the chemical action does not depend only on the *nutritive value* of the substance. This is especially clearly seen in the case of potassium nitrate, which acts repulsively, although it is a nutrient in many cases.

Apart from the *specific action* of the individual substance dependent on its chemical constitution, the *concentration* in which the solution is presented is of importance. *Mucor stolonifer*, for instance, reacts to a 0·1 per cent. sugar solution in a positive manner but more markedly to a 2 per cent. solution, and the reaction becomes more vigorous as the concentration is increased. At 15–30 per cent. the response is less apparent, and at 50 per cent. a repulsive reaction ensues. An exact determination of the critical concentration between positive and negative response is required, and experiment has yet to show what is the lowest concentration or liminal intensity which has a stimulating effect. The statements as to *repellent* concentrations are very meagre, because very often before repulsion takes place the plants have suffered injury. Miyoshi (1894a) records the following results, however:—a 10 per cent. solution of ammonium phosphate was repellent in the case of *Saprolegnia*, while a 5 per cent. was still attractive; a 3 per cent. solution of ammonium chloride (doubfully lower concentrations) was repellent to the same plant; a 50 per cent. solution of cane sugar in Hyphomycetes and a 20 per cent. in *Saprolegnia*; a 50 per cent. grape sugar in Hyphomycetes and a 10 per cent. in *Saprolegnia*; a 20 per cent. beef extract in *Saprolegnia* and some Hyphomycetes but not in Mucorinae. In the last case the action was doubtless due to the phosphates present in the extract. The liminal values of the attractive solutions as inducing stimulation were naturally lowest in the case of the best media. The liminal value of meat extract is 0·005 per cent. for *Saprolegnia*, of grape sugar 0·01 per cent. for *Mucor mucedo*, and of ammonium nitrate 0·05 per cent. for the same fungus. The determination of the liminal value for differences in concentration on opposite sides is obviously of greater importance than the determination of the absolute liminal value, which cannot be

determined exactly. How great must this difference be so that perception may follow, and how does this value vary with the absolute concentration? In order to obtain a definite but at the same time constant difference in concentration on opposite sides, MIYOSHI sowed spores of a fungus on a collodium membrane perforated in the middle, and laid it between two strips of filter paper crossing each other at right angles. If streams of different concentration were allowed to pass through the filter paper on both sides, the difference in concentration affecting the germ tubes was kept approximately constant. When *Saprolegnia* was made to grow between a 0·1 per cent. and 0·3 per cent. solution of sugar no curvature of the hyphae took place; the same result was obtained on using a 0·1 per cent. and 0·5 per cent., but when the solution on one side was 0·1 per cent. and on the other 1 per cent., positive chemotropic curvature took place. The same *relative* percentages must be maintained at higher concentrations if perception is to follow; thus a 0·5 per cent. solution of sugar must be opposed to a 5 per cent. MIYOSHI thought he was entitled to conclude from these experiments that in general the solution must be ten times as strong on one side as on the other if curvature was to take place (WEBER'S Law; compare pp. 473 and 543). Investigations must, however, first of all be made as to whether this relation holds good near the critical concentrations, and whether it is effective at high concentrations, where negative curvature appears. It is very probable that it is not so; moreover the repellent results obtained at higher concentrations are in all probability due, at least in part, to osmotic and not to chemical activity (MASSART, 1889; compare Lecture XLIII, p. 542).

In pollen-tubes as well as in Fungi, well marked capacity for responding to chemotropic stimuli has been established (MOLISCH, 1889, 1893; MIYOSHI, 1894 b; LIDFORS, 1899).

If we place a portion of a stigma, a style, or an ovule of *Scilla patula* on sterilized gelatine and sprinkle pollen of the same plant over the gelatine, keeping the whole preparation moist and in the dark, we find that the pollen-tubes invariably grow towards the tissue and finally pierce it. The fact that Fungi behave in the same way makes it very probable that what attracts the pollen-tube is nothing out of the common but merely some kind of sugar or other body commonly found in the plant. Since the stigma contains glucose, and since the ovule has been shown to contain a polysaccharide, we naturally think at once of cane or grape sugar as the exciting agent. As a matter of fact MIYOSHI (1894 b) has shown that pollen-tubes react vigorously to cane sugar and other soluble carbohydrates, such as levulose, dextrose, dextrine, and lactose, while the other substances which are active in the case of Fungi are in this case indifferent or repellent. MIYOSHI was also able to determine the liminal difference in concentration for pollen-tubes by the same method as he adopted in his experiments on Fungi. In the case of *Agapanthus*, chemotropic curvature always took place when the concentration of the stimulant was at least five times greater on one side than it was on the other, a fact which was established for percentages of 0·5, 1 and 2. Starting from this basis MIYOSHI was able to deduce the degree of concentration of the cane sugar solution which escaped from the ovules of *Hesperis matronalis*, assuming that the sensitivity of the pollen-tubes of this plant was exactly the same as of those of *Agapanthus*. When pollen-tubes and ovules were both placed on gelatine containing a known amount of cane sugar, and whose surface was also moistened with a sugar solution of the same concentration, approximation of the pollen-tubes to the ovules took place only when the secretion from the ovule was at least five times as concentrated as that in the gelatine medium. Growth towards the ovule took place only if the substratum contained 0·25 per cent. to 2 per cent. of sugar but not at higher concentrations, hence the concentration of the cane sugar solution in the ovule must have been at least 10 per cent.

It is well worthy of note that MIYOSHI has determined in a *large number* of

plants that an excretion of cane sugar takes place from the ovule and that the pollen-tube reacts to this substance. There can be no doubt therefore that the ovules of *one* plant must attract the pollen of entirely different plants, a fact which has indeed been definitely proved in many cases by experiment. Since, however, in nature entry of foreign pollen is prevented, other conditions must co-operate, especially the conditions necessary for the *germination* of the pollen, where chemical stimuli, often of a highly specific nature must play a special part (pp. 317 and 372). Further, we must not imagine that all pollen-tubes react *only* to sugars as stimulants. In this relation LIDFORS (1899) has recorded observations which have extended and completed those of MIYOSHI. He was able to show that in *Narcissus tazetta* the attractive substance was not a carbohydrate at all, and, after various attempts, he succeeded in proving that it was a *proteid* that induced the chemotropic reaction. The *decomposition-products* of the proteid, however, were quite inactive. [As to the chemotropism of roots compare SAMMET (1905), LILIENFELD (1905), NEWCOMBE and RHODES (1904).]

Hitherto we have considered only *liquid* or *soluble* bodies in relation to chemotropic activity; but it is obvious that *gases* may also have this effect, for they also diffuse and might affect different sides of plants if in different degrees of concentration. Chemotropic curvatures due to gases have, as a matter of fact, been observed by MOLISCH (1884) in roots and later in pollen-tubes (1893), phenomena to which he has given the name of *aerotropism*. MOLISCH's method of experimenting was as follows:—he separated two chambers from each other by a vertical plate, and placed different gases in each. The plate was pierced by a narrow slit, in front of which, at the smallest possible distance off he placed the radicle of a seedling; the opposite sides of this radicle were thus in different atmospheres. When the root was placed at the boundary between ordinary air and air poor in oxygen, a curvature took place towards the atmosphere which was richer in oxygen, and this capacity must obviously prevent the root from penetrating too deeply into the lower layers of the soil; in other words aerotropism is a factor in determining the depth to which roots penetrate the soil. *Negative* aerotropism to oxygen has also to be taken into account. This phenomenon makes itself evident when the root has to choose between an atmosphere of ordinary air and one composed of pure oxygen; the root bends towards that which is poorer in oxygen. By altering the oxygen concentration on both sides a condition is at last reached when neither positive nor negative curvature takes place, a condition of indifference in short so far as this gas is concerned. MOLISCH found that the root responded only *negatively* to all the other gases he investigated, viz. carbon-dioxide, chlorine, hydrochloric acid, coal gas, ammonia, and chloroform. When the concentration of these gases was increased, a *positive* curvature certainly often appeared, but that was merely due to injury to the concave side of the root, and it was no more a genuine stimulus reaction than the positive curvature already described as occurring in galvanotropic reactions. [MOLISCH's results have recently been called in question by BENNETT (1904); compare also SAMMET (1905).]

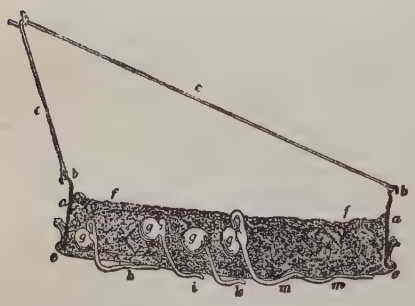


Fig. 152. Hydrotropism in the root. After SACHS (from DETMER's Smaller Practical Physiology).

Since, according to MOLISCH, the aerotropic movements took place after removal of the root apex, we must conclude that the act of perception of the stimulus takes place in the growing zone, and this constitutes a difference between aerotropic and *hydrotropic* curvatures, which have also been observed

on roots although in other respects the two series of phenomena closely resemble each other. As in the case of aerotropism we had to take account of perception of unequal distribution of a gas, so in hydrotropism we have to deal with unequal distribution of water vapour. SACHS (1872) demonstrated hydrotropism in the root in a very simple way (Fig. 152). He covered a shallow zinc cylinder on one side with a large-meshed netting, filled the cylinder with wet sawdust, and suspended the whole apparatus so that the perforated under side of the cylinder formed an angle of 30°–45° with the horizontal. Peas were then planted in the sawdust, and the roots on germination soon exhibited positive geotropism, growing out of the sawdust through the meshes into the air. 'If the air outside be completely saturated or nearly so, the roots grow straight out into the air; if the air be not saturated but only moderately damp, the roots bend sideways and curve over until they again reach the under side of the sawdust. Not infrequently they grow backwards closely adpressed to the oblique surface and sometimes the root apex re-enters the moist sawdust through the meshes, at once curving out again geotropically and repeating the performance several times thus lacing itself through the meshes.'

MOLISCH (1883) has shown that the stimulus in positive hydrotropic curvature is perceived by the *root apex*. He surrounded the root tightly with moist blotting paper so that only about 1 mm. of the apex was exposed. When a psychrometric difference was established, positive hydrotropic curvature ensued, just as when the growing region was subjected to dissimilar amounts of water vapour on either side. PFEFFER (1894) has more recently extended this research by showing that the stimulus is perceived *only* by the root apex. If the *apex be uniformly wet on all sides* hydrotropic curvature never occurs in the growing zone. Further experiments have yet to be undertaken to determine whether special emphasis is to be laid on this difference between aerotropism and hydrotropism, viz. the great senitiveness of the apex in the latter case and the absence of that sensitivity in the former.

We will only note further the fact that hydrotropism is by no means confined to the root. Positive hydrotropism is exhibited also by the rhizoids of the Marchantiaceae and negative hydrotropism by certain, but by no means all, plumules, e.g. *Linum* (MOLISCH, 1883), potato (VÖCHTING, 1902), (compare SINGER, 1903, Ber. d. bot. Gesell. 21, 175); negative hydrotropism is on the other hand of very general occurrence in Fungi (*Mucor*, *Phycomyces*, *Coprinus*). The sporangiophores of *Phycomyces* are remarkably sensitive to water (WORTMANN, 1881) and lead to quite special phenomena in this case. If *Phycomyces* be grown on bread, it may be noticed that the sporangiophores, though geotropic, do not, in darkness, stand perfectly erect; they form rather a tuft of diverging filaments. Each sporangiophore, owing to transpiration, renders the air in its immediate vicinity damp, and hence the neighbouring sporangiophores curve away from it, and since the same applies to all of them, the result is this outward divergence of the sporangiophores, the one from the other. The marked attraction of *Phycomyces* by certain insoluble bodies such as iron, which ELFVING (1890) attributed to physiological stimulation at a distance, is due to hydrotropism (ERRERA, 1892; STEYER, 1901). Iron is hygroscopic and hence the air in the neighbourhood of that metal is somewhat drier than before; *Phycomyces* bends therefore negatively hydrotropically towards the drier region. Obviously it reacts to very minute psychrometric differences, but exact experimental data on the subject are not as yet forthcoming.

The behaviour of *Phycomyces* when placed on a klinostat is also quite peculiar; the sporangiophores stand at right angles to the surface of the rotating medium, just as if there were a radially acting force emanating from it. In reality the position which *Phycomyces* takes up in relation to the substratum can be explained by hydrotropism only, since it alters when the air is *saturated*. At the same time it must be noted that an ordinary moist chamber is not ab-

solutely saturated, but that the saturation is always increasing sufficiently to induce directive movements on the part of the fungus. In the case of other plants, however, grown on a klinostat, e.g. *Lepidium sativum*, orientation with special reference to the substratum may easily be observed. If *Lepidium* be cultivated on a cube of turf the plumules arrange themselves at right angles to the four faces of the cube which are parallel to the axis of the klinostat, while they assume somewhat oblique relations to the two other sides, turning their convex sides to the axis. These positions cannot be explained by hydrotropism since the plumules of *Lepidium* are not hydrotropic. Probably we have here to deal with heliotropic movements which in the conditions of illumination under which the experiment is generally conducted are by no means excluded (DIETZ, 1880). In the dark such orientation in relation to the substratum completely disappears.

We may conclude this lecture by drawing attention to two other tropisms about which little is known. DARWIN (1881) was the first to describe *traumatotropism* in roots. If the growing point (SPALDING, 1894) of a root be injured on one side by some chemical or by heat, a curvature takes place in the growing zone, which has the effect of removing the end of the root from the injurious substance. Data with regard to this tropism are almost entirely wanting [compare BURNS, 1904]; we do not know whether the stimulus is to be sought for in some chemical change in the root or whether even the curvature itself has any purpose in nature.

We are better acquainted with the phenomena of *rheotropism* first described by JÖNSSON (1883) as occurring in roots, more especially in those of seedlings, but not limited to them. When such roots are grown in running water, they exhibit a curvature in the *opposite* direction to the course of the flow (positive rheotropism). NEWCOMBE (1902 a) found that in the case of the roots of Cruciferae, which respond best to this stimulus, the minimum rate of water flow which was capable of acting as a stimulus was 2 cm. per minute; the best results were obtained when the speed had reached 100–500 cm., and at about a 1,000 cm. negative curvature ensued, although these would appear to be due to purely mechanical causes and not to be stimulation phenomena at all. JUEL (1900) has made similar experiments with like numerical results for *Zea mais* and *Vicia sativa*. Rheotropism, however, is not a peculiarity exhibited by all roots, and the individual variations in different cases are, according to the statements of all investigators, very considerable (compare BERG, 1899).

More recently (1900) JUEL has shown that decapitated roots still react rheotropically, and NEWCOMBE (1902 b), considers it probable that the stimulus makes itself felt not only in the growing zone but also at the apex and in older parts up to a distance of 15 mm. from the apex. That rheotropism has nothing in common with hydrotropism, as one might at first sight imagine it had, has been shown experimentally by JUEL, who has proved that the immediate perception is due to the *pressure* of the flowing water. Hence rheotropic curvatures should be correlated with the movements in roots due to unilateral contact (Lecture XXXVIII).

We have now gained some insight into tropistic curvatures due to a large number of different stimuli, but we must not attempt to consider the combined action of *several* stimuli, as we attempted to do in the case of geotropism and heliotropism.

Bibliography to Lecture XXXVII.

DE BARY. 1884. Vgl. Morphologie u. Biologie d. Pilze. Leipzig.
[BENNETT. 1904. Bot. Gaz. 37, 241.]
BERG. 1899. Lunds Univ. Arsskrift, 35.
BRUNCHORST. 1884. Ber. d. bot. Gesell. 2, 204.
[BURNS. 1904. Beihf. bot. Centrbl. 18, I, 159.]

Czapek. 1895. Sitzungsber. Wiener Akad. 104, 337.
Czapek. 1898. Jahrb. f. wiss. Bot. 32, 175.
Darwin. 1881. Bewegungsvermögen d. Pflanzen. German ed. by Carus. Stuttgart.
Dietz. 1888. Unters. bot. Inst. Tübingen, 2, 478.
Elfving. 1882. Bot. Ztg. 40, 257.
Elfving. 1890. Commentationes var. Univ. Helsingfors.
Errera. 1892. Annals of Botany, 6, 373.
Hegler. 1891. Verh. d. Gesell. d. Naturforscher, 108.
Jönsson. 1883. Ber. d. bot. Gesell. 1, 512.
Juel. 1900. Jahrb. f. wiss. Bot. 34, 507.
Kihlmann. 1883. Act. Soc. Fennicae, 13.
Klercker. 1891. Oefvers. Vetensk. Akad. Förhandl. Stockholm, 10, 778.
Lidfors. 1899. Ber. d. bot. Gesell. 17, 236.
[Lilienfeld. 1905. Ber. d. bot. Gesell. 23, 91.]
Massart. 1889. Arch. de Biologie (van Beneden and Bambeke), 9, 515.
Miyoshi. 1894 a. Bot. Ztg. 52, 1.
Miyoshi. 1894 b. Flora, 78, 76.
Mohl. 1851. Die vegetab. Zelle, p. 140. Braunschweig. (R. Wagner's Handwörterbuch d. Physiologie.)
Molisch. 1883. Sitzungsber. Wiener Akad., Math.-nat. Kl., I. Abt. 88, 897.
Molisch. 1884. Ibid. 90, 111.
Molisch. 1889. Sitzungsanzeiger Akad. Wien.
Molisch. 1893. Sitzungsber. Akad. Wien, Math.-nat.-Kl., I. Abt. 102, 423.
Müller-Thurgau. 1876. Flora, 59, 65.
Newcombe. 1902 a. Bot. Gaz. 33, 177.
Newcombe. 1902 b. Annals of Botany, 16, 429.
[Newcombe and Rhodes. 1904. Bot. Gaz. 37, 23.]
Noll. 1892. Ueber heterogene Induction. Leipzig.
Pfeffer, quoted by Rothert. 1894. Flora, 79, 212.
Sachs. 1872. Arb. bot. Inst. Würzburg, 1, 209.
[Sammet. 1905. Jahrb. f. wiss. Bot. 41, 611.]
Spalding. 1894. Annals of Botany, 8, 423.
Steyer. 1901. Reizkrümmungen bei *Phycomyces*. Diss. Leipzig.
Van Tieghem. 1884. Traité de botanique. Paris.
Vöchting. 1888 a. Ber. d. bot. Gesell. 6, 167.
Vöchting. 1888 b. Bot. Ztg. 46, 501.
Vöchting. 1890. Jahrb. f. wiss. Bot. 21, 285.
Vöchting. 1902. Bot. Ztg. 60, 87.
Wiesner. 1878–80. Die heliotrop. Erscheinungen. (Denkschr. W. Akad.)
Wortmann. 1881. Bot. Ztg. 39, 368.
Wortmann. 1883. Ibid. 41, 457.
Wortmann. 1885. Ibid. 43, 193.

LECTURE XXXVIII

HAPTOTROPISM

At the end of the preceding lecture we referred to curvatures which occurred in the root as a consequence of *contact* stimulus. Such haptotropic or thigmotropic movements are most conspicuously illustrated by tendril-bearing plants (Darwin, 1876 a ; Pfeffer, 1885 ; Fitting, 1903 a) since these plants are provided with special organs or ' tendrils', whose function it is to attach themselves to supports by haptotropic curvatures. Just as in the case of the twining plants, the tendril-bearers are unable to stand erect by their own unaided efforts, and hence they make use of any rigid bodies available as supports, whether these be dead or alive. The attachment to the support is effected by a spiral winding of a tendril round it. Since the tendrils are generally either leafless lateral

branches or leaves without laminae, it may be said that support is effected by means of *lateral organs* while the chief shoot grows straight on. In this respect tendril-bearers differ markedly from most twining plants; but there is another and more important distinction, viz. that the twining stem can hold on from below upwards only to a more or less erect support and twine in a definite direction, i.e. to right or left, while tendrils can attach themselves also to horizontal supports and can twist round them to the right or to the left, upwards or downwards. This points to quite *different physiological properties* of these two closely *related biological groups* of plants—a point which will come out with perfect clearness in the following treatment of the subject.

Starting with typical tendrils, such as we find in the Leguminosae, Cucurbitaceae, or Passifloraceae, we find them to be long, slender, flexible structures, which, as in *Passiflora*, arise singly in the axils of the leaves, or which, as in the Cucurbitaceae, arise singly or in greater numbers on a tendril-bearer, alongside a leaf on the chief axis (Fig. 153. Compare GOEBEL, Organographie,

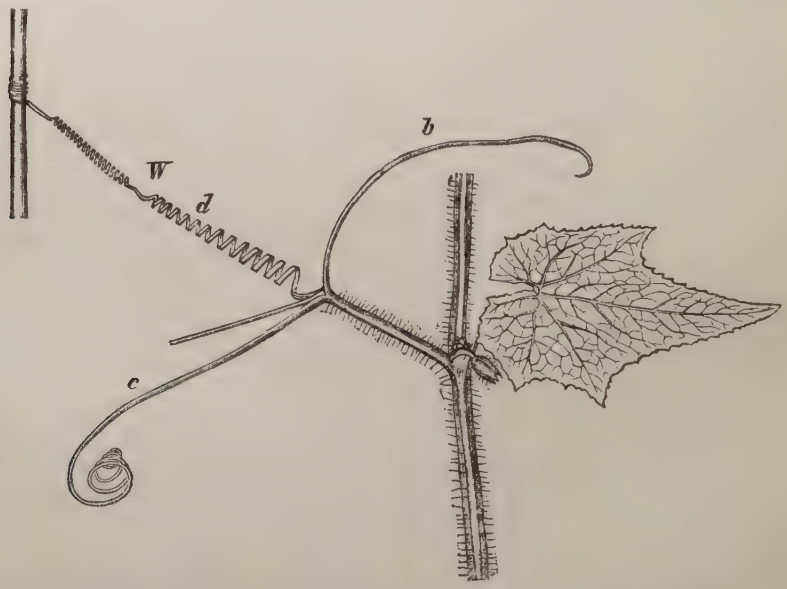


Fig. 153. Tendril-bearer of *Sicyos angulatus*. *b*, feebly stimulated; *c*, strongly stimulated; *d*, a tendril which has attached itself to a support. At *W* a reversion of the direction of twining has taken place. From DETMER'S Smaller Practical Physiology.

p. 610); in the Leguminosae the tendrils generally occur at the ends of leaves. A transverse section of a tendril is, as a rule, circular, but often it varies from that shape and becomes flattened. Commonly the anatomical structure is markedly dorsiventral (O. MÜLLER, 1887); it is possible to distinguish an upper and an under side and a right and a left flank. Even if, anatomically, there be no difference between the sides it is possible to recognize dorsiventrality by other evidence, for very frequently the tendrils, as they develop from the bud, are rolled up in a spiral manner and the convex side is then always the *under side*. They begin to grow rapidly and in a few days reach their definite length by straightening. During this period they perform peculiar movements, i.e. of circumnutation, which recall those of the twining plants, but which are of a purely *autonomous* character and which, for that reason, cannot be discussed in this connexion (Lecture XLI). It may be noted only that the tendril, in consequence of the fact that *one* side is growing more rapidly than the other, is slightly bent

and that it describes approximately a conical curve in space, because the zone of more vigorous growth affects successively new surfaces. At first the axis of the cone is directed steeply upwards, but it gradually sinks lower and lower below the horizontal, in which position finally the circumnutations cease. The growth which now takes place is very conspicuous, for the whole tendril, under suitable conditions, may elongate from 50 per cent. to 90 per cent. in one day, and individual zones may show extension amounting to 100 per cent. Growth is intercalary, for no apical elongation takes place. It is most vigorous in the basal half and continues in that region for a longer time than in the apical half. After about 3 to 5 days the tendril *appears* to be full-grown, but more accurate measurements show that it is still growing though feebly, i.e. about 0·5–2 per cent. in 24 hours. After this feeble growth has continued for a few days vigorous growth recommences, although it never reaches its original activity. The growth moreover is always *unilateral*, the *upper* side growing more rapidly, and hence inducing the formation of a coil or spiral, the concave side being the under side morphologically. This spiral coiling begins as a rule in the middle or in the basal part of the tendril and spreads towards the apex.

The specific sensitivity of the tendril, the capacity for twining round a support, makes itself apparent as soon as it has reached from $\frac{1}{4}$ to $\frac{1}{3}$ of its ultimate length, and it may be still observed when spontaneous inrolling takes place. The sensitivity ceases when the tendril is full grown. In order to understand how the enclosure of the support by the tendril is achieved, we must study first of all the incurving movement which takes place after a brief contact with some solid body. If, for instance, the tendril of *Passiflora* or of *Cyclanthera pedata* be rubbed on the *under side* with a splinter of wood or a pencil, after a few minutes, or even a few seconds in some cases (*Cyclanthera*, 5–7 seconds ; *Passiflora* and *Sicyos* 25–30 seconds), a vigorous incurving takes place, the under side to which the friction was applied becoming concave, and this incurving progresses so rapidly that it is often possible to follow the movement with the naked eye. After 30 seconds, or a longer period in less sensitive tendrils, the curvature ceases and soon thereafter the tendril becomes straight once more, but the *undoing* of the curvature always takes a much longer time than its *formation*.

So far as the result is concerned it is by no means immaterial to which part of the tendril the friction is applied ; we find that the under side is not equally sensitive throughout its entire length. The same stimulus produces a more rapid response if applied to the upper third of the tendril than if applied in the middle, and no visible response is given by the base of the tendril where the growth is most vigorous. On the flanks the sensitivity also decreases from the base to the apex, and it is generally less there than on the under side. Further, on the flanks the stimulated part is always towards the concave side, but the curvature is less marked and slower than when an equally strong stimulus is applied to the under side. Finally, if the upper side be stimulated, as a rule no curvature follows. This is not the case, however, with *all* tendrils; those of *Cobaea scandens*, *Eccremocarpus scaber*, *Cissus discolor*, &c., curve quite as vigorously when the dorsal as when the ventral side is stimulated. We shall find later on that these tendrils also are physiologically dorsiventral. Such tendrils as those of the plants just named may be considered as uniformly sensitive on all sides, as contrasted with the others previously referred to, which were sensitive on one side only (Darwin, 1876a). Fitting (1903a) had shown, however, that the dorsal side of unilaterally sensitive tendrils can receive a stimulus although it does not respond by curving. The facts on which he bases this conclusion are as follows : If a tendril be touched with a stick on a reacting side only a short distance away an incurving takes place *only* at the stimulated place, and this curving is propagated about 2–5 mm. on either side of the region of stimulation. If *two* places be stimulated about 1½–2 cm. apart, two curvings occur, the region between remaining straight. If the *whole* of one side of the tendril be stimulated from base to apex, the tendril

rolls itself up into a spiral, from the apex downwards. If a tendril which is sensitive on all sides be stimulated first on *one* side and, *at the same time* or *shortly afterwards* on the opposite side, no curvature results, provided the stimulus on one side be equal to that on the other. If the same experiment be tried with a tendril sensitive on one side only and if *both* upper and under sides be stimulated, one would expect that the response would be the same as if the under side only had been stimulated. That is, however, by no means the case, for the tendril remains quite straight. If a small part of the upper side and at the same time the whole of the under side be stimulated, curvature takes place only at the places on the under side which lie opposite to the unstimulated regions of the upper side. The *sensitiveness* to contact is thus as well developed on the upper as on the under side, and the difference between the two sides lies in the fact that while stimulation of the under side induces *curvature*, stimulation of the upper side induces *no visible result* or simply inhibits curvature on the under side, according to circumstances. Following FITTING, we must therefore recognize tendrils which *react equally* on all sides and tendrils which do *not* do so. As to the behaviour of the latter type we shall have a clearer conception after we have analysed the stimulus movement into its different phases. First of all let us examine the nature of the perception of the stimulus.

On this subject DARWIN has already made certain statements. He assumed that the tendrils reacted to a definite *pressure* and therefore laid light weights, such as wires, threads, &c., on the parts of the tendril capable of movement, taking the utmost precautions against inflicting a shock. He found that the tendril of *Passiflora* gave curvature-response to the pressure of a piece of platinum wire 1·23 mg. in weight, and to a piece of cotton thread 2·02 mg., while the tendrils of other plants required greater weights, up to 4–9 mg., before any response could be recognized. According to PFEFFER (1885), to whom we owe an elaborate investigation into the phenomena of perception of contact stimulus by tendrils, these experiments of DARWIN'S do not meet the case at all, since as a matter of fact much greater *pressures* may be exerted on the plant without any visible response resulting. A continuous or statical pressure generally never induces curvature, and even in DARWIN'S experiments, in spite of every care being taken, vibrations could not be avoided when the weights were placed on the tendrils or afterwards. The *shocks*, slight as they were, operated as a stimulus. If *shocks* be not eliminated, *far lighter* bodies than those employed in DARWIN'S experiments will induce curvature, e.g. a particle of cotton thread 0·00025 mg., which was placed in position simply by a draught of air. On the basis of PFEFFER'S researches the perceptive capacities of tendrils may be estimated in the following way.

We may first of all inquire whether liquids as well as solids may act as stimuli to the tendrils. This is obviously not the case, since if we inflict only very slight blows on the tendrils with a solid body curvature appears at once, while an equal shock from a liquid never induces any reaction. PFEFFER allowed water, watery solutions of various substances, oil, and finally mercury to strike the sensitive region of the tendril at greater or less velocities and obtained no reaction, although the mercury had an injurious effect on the tendril. These facts are of great importance, for they show that tendrils cannot be stimulated by raindrops ; a capacity for reacting to such stimuli would be obviously quite meaningless. If, however, there be any small solid particles in the liquid, such as crystals in the oil, mud in the water or accidental impurities in the mercury, stimulation is at once set up. It would appear therefore that tendrils are able to discriminate between different conditions of aggregation of bodies and to react to the solid but not to the liquid. Yet this is by no means the case, for tendrils are unable to distinguish gelatine containing 10–14 per cent. of water from a liquid, although that substance is *solid* at ordinary temperatures. This fact suggested a whole series of interesting experiments, for PFEFFER was enabled,

first of all, by employing a 14 per cent. gelatine solution which was kept moist, to fix bodies to the tendrils under investigation without inducing stimulation ; further the gelatine was smeared over a glass rod and this rod was employed in the study of the influence of different types of contact on tendrils. By its means the effect of constant pressure, both uniform and gradually increasing, was tried ; solitary or numerous successive blows, light or heavy, were inflicted on the tendril, or the tendril was rubbed with the rod ; *in no case*, however, *did any stimulation occur*, nor did any curvature follow after constant pressure or blows, single or successive, or after friction; each and all were quite ineffective.

In the course of his experiments PFEFFER proved that blows, administered by a solid body (apart from gelatine) were *stimulants* provided they were of sufficient intensity. Thus thin smooth glass threads, sticks of wax, filter paper, animal membrane in the dry and the wet condition induced a positive reaction, and the significance of the velocity of the impacts could be easily demonstrated by means of particles of clay suspended in water. On the other hand it was seen that solid bodies induced no reaction if the pressure they exerted were *statical*, that glass threads or needles, if they were pressed against the tendrils cautiously and without any friction and without any sudden increase in the pressure, produced no effect. Nor was there any result when a short piece—about 4 mm. long—of the tendril was subjected to a constant pressure of a solid body, on which there were several different points of contact (e.g. a rusty nail, emery paper). In all these cases, however, a reaction took place at once when the bodies in question were gently rubbed on the tendril no matter how limited the surface of contact was. Of the greatest importance is the fact that small blows of this kind, though unable to induce a reaction when administered *singly*, induced curvature by summation of stimuli. The tendril remains sensitive for a remarkably long time to constantly recurrent stimuli, and while the reaction is in full progress new stimuli may be perceived until gradually the tendril becomes accustomed to them.

As a result of his inquiries PFEFFER summarized the perceptive powers of tendrils in the following words : ' In order that a stimulus may be effective *definite points of limited extent* in the sensitive region of the tendril must be affected by a push or a pull of sufficient intensity, simultaneously or in adequately rapid succession. On the other hand, the tendril does not react as soon as the blow affects all points of a larger surface with approximately equal intensity in such a way that compression of closely adjacent regions is sufficiently nearly uniform' (gelatine). Thus it is that tendrils are not excited either by mechanical shaking in general or by rain. ' Under all conditions a local compression, decreasing with sufficient rapidity, is a condition of stimulation, which is not induced by statical pressure only, even if such a pressure affects widely separated parts with considerable intensity.'

In order to have a single word to express the mechanical conditions of tendril stimulation we will use PFEFFER'S term, *contact*, although that word might be more appropriately applied to statical pressure ; we may say, in a word, that tendrils possess contact sensitivity. We shall find that other bodies as well as tendrils possess the capacity of responding to contact-stimulus. Whether or not a similar mechanical influence leads to perception in the interior of the cells in the case of geotropism, it is impossible at present to say ; there, apparently, *statical* pressure does act as a stimulus.

We must assume that the perception of the stimulus takes place in the *epidermis*, and PFEFFER has shown in this relation that the deformations of the protoplasm necessary for perception might be simplified in many tendrils by certain histological arrangements such as the so-called 'sensitive pits'. Still it must be remembered that there are many sensitive tendrils which do not possess such histological differentiations, so that these pits cannot obviously be a necessary condition of stimulation. It is needless therefore for us to

discuss them at length, and we may content ourselves with referring to HABERLANDT'S (1901) exhaustive memoir on the subject. HABERLANDT, in addition to anatomical data, records certain physiological arguments as to the nature of contact sensitivity. He points out that a vigorous radial pressure, a compression of the protoplasm, does not lead to a stimulation, and that the stimulus-movement is induced rather by *tangential* tensions in the protoplasm. Whether these observations help to explain the nature of the phenomenon we are not prepared to say.

It is very remarkable that tendrils which possess so great a capacity for distinguishing different mechanical influences should also react to stimuli very different in character from those of contact. We are indebted to CORRENS (1896 a) for proof of the fact that tendrils exhibit curvatures, when subjected to sudden changes of temperature (cooling or heating), quite similar to those induced by contact. As far as we know (FITTING, 1903 a, 614) the mechanics of growth are the same as those seen in contact curvatures. Further CORRENS has shown that *chemical* stimuli also induce curvature in tendrils, as for instance when they are treated with such diverse substances as iodine solution, acetic acid, chloroform, or ammonia. Finally PFEFFER has succeeded in obtaining responses by employing weak induction currents as stimuli.

We shall return to these phenomena at the close of this lecture, but we must not omit to draw attention to them here by way of showing that the sensitivity of tendrils is not so restricted as one might think from the publications on the subject. On the other hand curvatures due to heat or chemical stimuli may be the more readily disregarded since these are entirely unnatural and do not affect tendrils in the wild state.

Let us now turn to the *curving* itself that generally follows on the application of a stimulus. The extraordinary rapidity of the process has not unnaturally led to the assumption (DARWIN, 1876 a; MACDOUGAL, 1896) that the movement was due to a diminution of turgidity on the concave side, afterwards rendered permanent by growth. From FITTING'S (1903 a) researches it would appear, however, that turgor has no special part to play in the process, which is rather to be attributed to special *growth* phenomena. FITTING showed, by making microscopic measurements of the movements of ink-lines made at appropriate distances apart on the upper and under sides, that immediately after the stimulation the marks on the convex side separated much more rapidly than before. The elongation may be so great in the course of a few minutes as to amount to an increase of 50, 100, or even 160 per cent. in an hour, values not attained by the non-stimulated tendril in 24 hours, and this, too, independently of the age of the tendril. At the same time the indices on the under or concave side approximate somewhat, resulting in an absolute contraction of about 1 per cent. per hour. From these measurements it is obvious that not only do all the tissues of the stimulated zone lying between the axis (middle line) and the convex outer surface suffer extension, but that most of those towards the concave side also participate in the growth increment, in other words, the neutral line, which is neither elongated nor contracted, lies on the concave side of the medulla; the middle line itself exhibits a marked *increase* in growth. That can only be definitely established by direct measurements on the flanks which suffer elongation to the same degree as the middle line.

We *cannot*, however, draw any conclusions from these calculations as to whether the growth acceleration, which reaches a maximum at a point on the other side opposite to the point of contact, is the first and the only factor leading to curvature, or whether an inhibition of growth on the concave side takes place at the same moment or just previously. The observed approximation of the indices on the concave side may be *active*, or they may be caused passively by compression induced by the curvature. It is more probable that the growth *acceleration* is not active in all the parts which it affects but that the deeper

layers behave passively in relation to the peripheral layers where vigorous growth takes place. Unfortunately space will not permit of our discussing the question at greater length (FITTING, 1903 a, p. 615).

Some time after the completion of the curving a cessation of growth takes place and thereafter begins, as we have already seen, a straightening of the tendril. Measurements show that the hitherto convex side remains quite unaltered while the movement of the indices on the concave side indicate that growth is taking place, less vigorously it is true than on the convex side during the period of curvature, but still so as to show that a marked acceleration in growth is occurring as contrasted with that exhibited by the unstimulated tendril. This acceleration can be traced beyond the middle line. A graphic representation of these phenomena is given at Fig. 154.

The recorded facts show in the clearest manner that the whole movement following on stimulation is exceedingly complicated, since the curvature is not effected by a simple contraction of the stimulated side but by an *acceleration of growth* which is most marked at the spot on the convex side directly opposite to that on the concave side where the stimulus has been applied. Apparently therefore *perception is followed by a transmission of the stimulus.* Perception, transmission and reaction follow each other in this case with a rapidity which is very much greater than we have seen to be the case in any of the tropistic movements previously discussed.

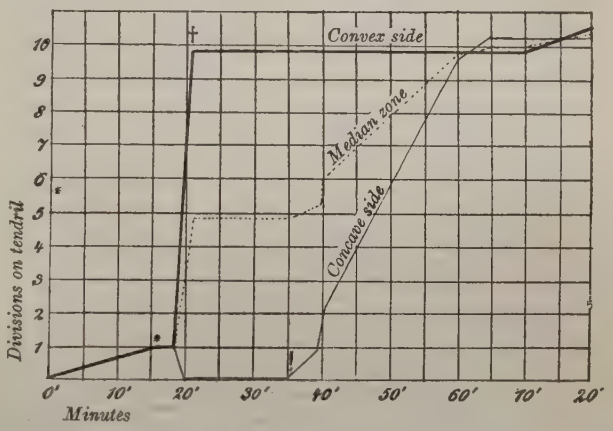


Fig. 154. Graphic representation of the growth of a tendril of *Sicyos angulatus*. *, beginning of the stimulation; †, completion of the curvature; !, beginning of the readjustment.

If we now trace the growth in a tendril which has been stimulated equally and simultaneously on both sides, we shall find that we always obtain the same result, whether it be a tendril which reacts on one side only or on all sides equally. *The tendril grows on as if nothing had happened to it*; increased growth, more especially, is absent altogether. We must therefore conclude that the absence of curvature when both sides are stimulated is not, as might be imagined, more especially in the case of tendrils which react equally all round, to be explained by assuming that the two stimuli induce two similar reactions on opposite sides. On the contrary one stimulus inhibits the effect of the other, and this is the case even when curving has already commenced. This fact shows that stimulation of the upper side does not by any means render *perception* on the under side impossible, but whether the inhibition affects the transmission or the reaction we cannot say.

There remains for consideration the significance of the backward curvature which takes place after each incurving. This is due doubtless to *internal* causes, for it is not a consequent of the contact stimulus, but primarily of the reaction for it follows, in virtue of growth acceleration, after every curvature induced by *purely mechanical* means. That it is a case of autotropism naturally suggests itself, such as we have already become acquainted with in considering the counter

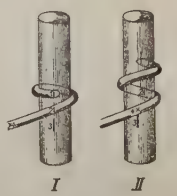
movements in geotropic curvatures. We have regarded the compression of the concave side there as a cause of this autotropism (BARANETZKY, 1901) and therefore the second growth acceleration in the case of tendrils could only take place if actual *curvature* had previously been effected. FITTING (1903) showed, however, that in tendrils also which are mechanically prevented from carrying out their curvature, there are likewise two temporary growth accelerations separated by a period of rest. It is very desirable that measurements should be taken of the distribution of growth during the period when curving is *ceasing*, since in those structures which are affected by a geotropic stimulus, a growth acceleration manifests itself in the median zone (compare p. 435); it is possible that the same relations may obtain in the present case. Meanwhile it is impossible to say with certainty whether the autotropism of tendrils is of a different nature or not from that already observed in cases of geotropism. If both phenomena be identical then the cause of autotropism must lie in an effort to curve, a *tension* of the parts concerned, and not in the first instance in the completed curvature (compare FITTING, p. 612).

Having now studied the movements of tendrils which result from a temporary contact, let us turn next to problems connected with the actual encircling of the support by the tendril in nature. The circumnutation of the tendril, exhibiting as it does movements very similar to those seen in twining stems, must aid it considerably in its efforts to find a suitable support; we might almost say that the tendril feels round about for a support to attach itself to. If the haptotropically reacting region comes in contact with some solid body the further nutatory movements enable it to place itself under conditions in which the haptotropic stimulus may operate. As in the experiments described, the tendril *rubs* itself against the support, and forthwith at once curves, whereby new regions of the tendril come in contact with the support. Since the stimulus, as we have seen, is transmitted for a few millimetres on either side of the point of contact, the tendril in a very short time describes a complete circle, provided that the support be not too thick or too thin. If the support be of suitable diameter the tendril is still unable to carry out completely the curvature striven after; a tension arises which exerts a pressure on the support, a tension which may be easily demonstrated by using compressible material, such as a roll of paper, as the supporting structure; such a substance will be found to have been squeezed by the tendril. The first coil is followed by a second and a third, if the tip of the tendril be still free. How comes it that these coils, it may be asked, remain *permanently* encircling the support if each incurving be followed by a recurving of the tendril? The reverse curvature may indeed be observed in tendrils which have managed to grasp a support, manifesting itself in a *loosening* of the existing coil. When this reverse curvature appears, however, the movement of the tendril or of the support may bring about a fresh contact stimulus, which again induces incurving, so finally effecting a permanent encircling of the support. So long as no loosening of the coil takes place, no new contact stimulus is possible, for the *pressure* exerted on the support does not act as a stimulus. As we have already seen, the stimulus is transmitted from the point of contact both proximately and distally. For purely mechanical reasons the base of the tendril cannot exhibit any curvature, since both ends are firmly fixed, to the plant on the one hand and the support on the other. But if the spiral on the support becomes slack, then curvature ensues in the neighbouring portions of the tendril basally, so that these come in contact with and surround the support, pushing in front of them the previously formed but now loose coils. A glance at Fig. 155 will make this clear. When the coils have succeeded in obtaining a permanent hold on the support further basipetal curvature ceases.

After the completion of the permanent twining, growth in length completely ceases, and there appears not only in these coils but also in the remainder of the tendril a number of important changes, which are apparently induced not by

contact but perhaps by *pressure* against the support. Longitudinal growth in the basal region comes rapidly to a standstill, although the tendril may by no means have reached the length attainable if a support be not grasped. Further, a spiral twisting manifests itself in the basal region, which is of great service to the plant inasmuch as it is the means of drawing the branch closer to the support. For purely mechanical reasons this intermediate spiral changes its direction at least *once*, but several such reversals of the spiral may frequently be noticed (Fig. 153 *W*). That this reversal is mechanically necessary, may be readily demonstrated by attempting to produce a spiral coiling in a cord or piece of rubber-tubing *fixed at both ends*. This spiral coiling recalls the autonomous curvatures already noted as occurring in older tendrils that have found no supports, since it always arises by excessive growth on the upper side. There are good reasons for believing, however, that the two phenomena are not identical, since intermediate spiral coils occur, after the clasping of the support, in tendrils which exhibit no free senile coiling (*Vitis*, DARWIN, 1876 a). [FITTING (1903 b) has shown that the incoiling of the base of the tendril after attachment to a support is a stimulus phenomenon which takes place in consequence of a growth acceleration in the median region.]

The following additional changes taking place in tendrils which have succeeded in finding a support, may also be noted. Not infrequently secondary thickening accompanied by a large development of sclerenchyma appears not only in the part immediately in contact with the support but also in the basal region of the tendril as well, which renders the tendril more capable of fulfilling its function (TREUB, 1882–3; EWART, 1898). Some tendrils also give off a secretion which helps them to adhere (O. MÜLLER, 1887); as to these and other morphogenic results of contact stimulus compare p. 315. Finally we must note that functional tendrils remain alive for a much longer time than those which have been unsuccessful in finding a support; the latter soon die, wither, or are thrown off.



I *II*

Fig. 155. *I*, tendril whose tip has just begun to coil round a support. Ink marks have been drawn, in a vertical direction, on the support and on the tendril, on the former at 3, on the latter at 1 and 2. Another line has been made at 4 on the tendril, at a place as yet at some distance from the support. *II*, the same tendril some hours later, showing the position of the original coil in *I*; the point marked 4 is now in contact with the support. (After FITTING).

Haptotropism manifests itself not merely in tendrils serving as specific climbing organs and which have entirely relinquished their previous functions and become adapted specially for that purpose, but it is manifested also in other organs which have remained functional so far as their main duties are concerned, but which add as a subsidiary function that of acting as climbing organs. Thus, for instance, ordinary roots may become sensitive to contact in their growing regions and may respond with haptotropic curvatures (SACHS, 1873, 436; [NEWCOMBE, 1902; NĚMEC, 1904]); and this capacity is so prominent in many aerial roots that we may even speak of them as 'root tendrils' (for literature see EWART, 1898). Contact sensitivity is also very prominently developed in leaves and especially in petioles (DARWIN, 1876 a). Plants belonging to many different families, e.g. *Clematis*, *Maurandia*, *Lophospermum*, *Tropaeolum*, *Solanum jasminoides*, &c., grasp supports by means of their petioles, while the leaf blades continue to act purely as assimilatory organs. *Fumaria officinalis* also climbs by means of unaltered laminae. *Nepenthes* may also be referred to in this connexion, where a certain part of the leaf functions as a tendril, while of the remainder, one part assimilates carbon-dioxide and one part acts as an insect-trap. *Lophospermum* may be specially mentioned as an example of a climber whose principal axis is sensitive to contact and twines round a support and in addition possesses both sensitive petioles and internodes. None of the examples cited have as yet been fully investigated from a physiological stand-

point, more especially as to whether they may be compared with genuine tendrils so far as the mechanics of curvature and their irritability are concerned. Into this question we need not enter.

A brief reference may now be made to *Cuscuta*. This remarkable plant is of special interest as forming an intermediate link between tendril-bearers and twiners. According to PEIRCE's researches (1894), *Cuscuta* has two regularly alternating conditions ; in the first it twines in a left-handed manner and, as a result of rotatory movements, the shoot apex forms a number of steep spirals round some vertical support only. After a time the second phase sets in, during which the plant behaves like a tendril, twining round its support in much less steep and tighter coils. These coilings are induced by contact with solids but not with moist gelatine, so that the sensitivity of the stem is obviously quite comparable to that exhibited by tendrils. In contrast, however, to tendrils, *Cuscuta*, during the time when it is sensitive to contact, is also geotropically active as well, and hence twines round vertical supports only. It may be noted also in passing that *Cuscuta* is capable of forming haustoria as a consequence of contact stimulus.

Haptotropic movements are also manifested freely by carnivorous plants,

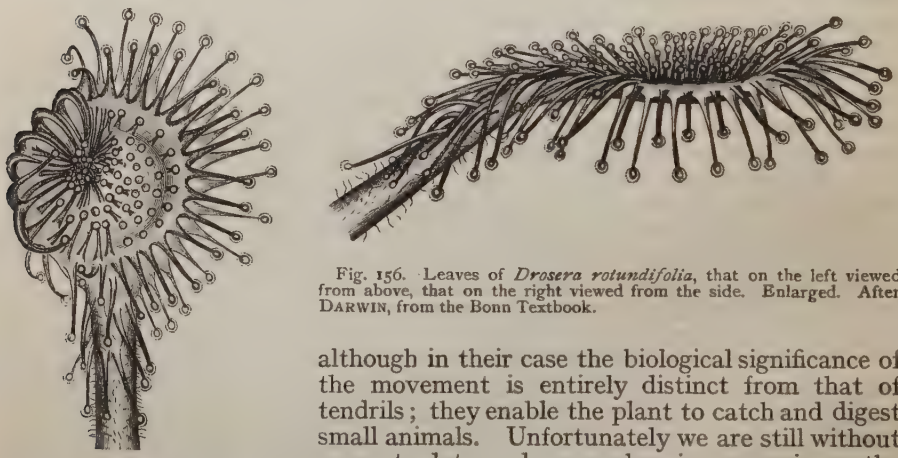


Fig. 156. Leaves of *Drosera rotundifolia*, that on the left viewed from above, that on the right viewed from the side. Enlarged. After DARWIN, from the Bonn Textbook.

although in their case the biological significance of the movement is entirely distinct from that of tendrils ; they enable the plant to catch and digest small animals. Unfortunately we are still without an up-to-date and comprehensive memoir on the nature of the movements in carnivorous plants, so that our knowledge on many important questions is very incomplete. We shall confine ourselves exclusively to the study of the leaves of *Drosera* (DARWIN, 1876 b). In the common native species, *Drosera rotundifolia*, the leaves are almost circular, attached to the axis by long petioles. The upper side of the blade is somewhat concave and studded with tentacles. The tentacles in the middle of the blade are glandular, shortly stalked and stand erect, while those at the periphery are long stalked and bent outwards. Each tentacle is tipped by a drop of sticky secretion which sparkles in the sun like a dewdrop—hence the popular name of the plant, 'sundew.' Small insects which chance to alight on the leaf are held fast by the sticky secretion, and by further excretion from the glands digestion of the body is effected. (Compare p. 184.) When a single tentacle has been touched by an insect, not only that one, but other tentacles also exhibit curvatures, until finally nearly all the glands come in contact with the prey and aid in digesting it. Let us assume that the insect touches one or more of the shorter tentacles in the centre of the lamina, these we shall find remain erect, but the stimulus is transmitted from them outwards, so as to induce an inward radial curvature of every peripheral tentacle. If the insect be caught by a peripheral tentacle, the latter only at

first proceeds to carry its booty towards the middle of the leaf, but as soon as it reaches the centre a stimulus is transmitted to the other peripheral tentacles which then begin to incurve also. We have thus to discriminate between movement induced by a direct stimulus from that resulting from a transmitted stimulus, and we are able to establish the fact that that transmission can be effected only by means of the central tentacles. All the disk tentacles, not merely those which occupy the exact centre, are able to radiate impulses. If the leaf be stimulated half-way between the centre and the margin at two opposite points, half the tentacles bend towards one centre of stimulation, half towards the other, so that, as DARWIN says, *two wheel-like* arrangements are formed, one on either side, whose spokes are formed by the tentacles and whose hubs are the points towards which all the glands concentrate. Let us now study the effect of a *direct* stimulus on a single tentacle, so as to obtain some idea of the nature of the sensitivity, the perceptive region, and the method of curvature.

Drosera reacts both to mechanical and chemical stimuli. The actively mechanical stimuli are contact stimuli as in tendrils. DARWIN (1876 b) showed that neutral liquids, such as water, produce no effect, although they are driven against the tentacles with considerable force, and PFEFFER (1885) also proved that a rod covered with gelatine (as in the case of tendrils) was ineffective. On the other hand, solid, insoluble bodies, even though extremely light, induce movement, provided only they were capable of penetrating the secretion. The tentacles may be stimulated also by a blow from a pencil or a splinter of wood, although to induce a result several successive contacts are necessary. In all these respects *Drosera* agrees entirely in its behaviour with tendrils. We owe to DARWIN the proof of the fact that the sensitivity is localized in the *gland* exclusively, and HABERLANDT (1901) showed that histological structures similar to those which occur in tendrils, i.e. sensitive pits, occur also in the epidermis of the tentacle. Curvature is entirely confined to the *stalk* of the tentacle and more especially to its base, which bends sharply while the upper part remains quite straight. This is most marked in the long-stalked peripheral tentacles (Fig. 157). As to the mechanics of the curvature we as yet know nothing. Since, however, BATALIN (1877) has shown that growth acceleration takes place during the curving, it is very probable that not only the nature of the sensitivity but also the mode in which the movement is carried out corresponds to that exhibited by tendrils.

Fig. 157. Part of a transverse section through a leaf of *Drosera*, showing three tentacles, one of which has undergone curvature as the result of a stimulation. After DARWIN (1876 b).

The tentacles respond very rapidly to a mechanical stimulus. After 10 seconds DARWIN was able, with the aid of a lens, to observe the commencement of curvature; curvature visible to the naked eye was often apparent in less than a minute. As bending proceeds the end of the tentacle in a very short time describes a considerable curve in space, the peripheral tentacles being able not infrequently to curve through an angle of 270° in the course of an hour. Some time after the completion of the incurving a reverse curvature takes place and a straightening of the tentacle, even if contact with the body acting as a stimulant is continued. This movement certainly takes place much more slowly than in the case of the tendril—according to DARWIN, in about 24 hours—yet the factors concerned and the mechanics of the movement would appear to be identical with the analogous processes in tendrils. Immediately after this straightening —possibly even sooner—the tentacle is ready to receive a new stimulus.

It has already been pointed out that *Drosera* is also capable of being stimulated by *chemical* agents. Chemical stimuli as a rule act much more vigorously than mechanical, as is shown by the rapidity of the movement and the *duration*

of the incurving. Apart from that, the curvature induced by chemical stimuli corresponds exactly to that induced by mechanical stimuli. DARWIN proved that many substances, varied in their character, acted as stimuli, when solutions of these were placed on the leaf of *Drosera*. Among these he found that many were neither useful nor yet injurious to the leaf, while, on the other hand, well-known poisons, such as corrosive sublimate, and even nutrients, like many ammonium salts or phosphates, are readily absorbed, or like proteid and proteinaceous animal compounds, were first digested by the secretion given off by the glands. Among the substances which were observed to be indifferent from the nutritive standpoint, distilled water may be mentioned more especially, whose value biologically as a stimulant is, according to CORRENS (1896 b), most perplexing. It must be especially noted that in CORRENS'S experiments the chemically purest water possible was employed; curvature is not induced if traces of soluble substances be present. Natural water is *not* a stimulant and that because the fact that it contains lime inhibits the sensitivity of *Drosera* as do such anaesthetics as ether, chloroform, &c. (compare p. 98).

Let us now glance at the curvatures induced in the tentacles by *transmitted* stimuli. Very little is known as to the transmission of the stimulus itself; it has not been determined whether the agent in transmission is the parenchyma of the leaf (DARWIN, 1876 b) or the vascular bundle (BATALIN, 1877). So far as we know the stimuli are transmitted exclusively from the tentacles in the centre to those on the outside and not vice versa. The impulse thus transmitted acts directly on the motile zone from beneath; that it need not first of all be transferred to the apex may be concluded from the fact that decapitated tentacles (DARWIN, 1876 b, p. 219) react to a transmitted stimulus, although they are not *directly* sensitive. This fact, in addition to its general significance, has a special interest, inasmuch as it enables us to interpret a certain phenomenon seen in the sap of the tentacular cells of *Drosera* which has been spoken of as 'protoplasmic aggregation'. If the apex of a tentacle be stimulated its cells exhibit certain peculiar alterations. Protoplasmic movements first of all take place (DE VRIES, 1886), and the single vacuole breaks up into a large number of small ones. These proceed to contract by apparently ejecting some of their contents, e.g. sugars and organic acids. These vacuoles, surrounded by their protoplasmic membranes, lie in the extruded cell-sap; the vacuoles contain a red colouring-matter and hence the whole process may be readily followed. The aggregation progresses from the stimulated gland down the tentacle from cell to cell and appears later on in the other tentacles which have been indirectly stimulated. We might interpret this progressive aggregation as the visible expression of the transmission of the stimulus. More accurate investigation, however, reveals the fact that the aggregation appearing in the indirectly stimulated tentacles does not commence simultaneously with the curving but only when the *glands* begin to secrete. Further, in the indirectly stimulated tentacles the aggregation progresses from above downwards not from below upwards, i.e. in the path of the stimulus. Hence it is obvious that aggregation is a process closely connected with secretion and not with curvature, and we further know that it is associated with very remarkable changes in the nuclei of the gland-cells during secretion (ROSENBERG, 1899). It is also worthy of note that, according to DARWIN (p. 220), much feebler aggregations are exhibited by decapitated tentacles.

Apart from the fact that when the tentacle is directly stimulated the stimulus is transmitted only *from above* downwards to the motile zone and in indirect stimulation *from below* upwards, there is another and more important difference between the two kinds of stimulation. In the former case it is always a predetermined side of the tentacle (the outside) that becomes convex, while in the latter case the course of the resulting curvature is determined by the direction from which the stimulus comes (p. 497). Either flank of the tentacle

may become convex, and it has yet to be determined whether the inner side can be rendered convex under appropriate experimental conditions. It is only as a result of indirect stimulation that a *tropistic* curvature is induced, the curvature in the other case is nastic. By *nastic* curvatures we mean (compare p. 428) those whose direction is determined by the plant itself. A nastic curvature may, however, be induced just as well by a stimulus directed in a definite direction as by diffuse stimuli. Which process takes place in *Drosera* is not quite established, but it is very probable that the stimulus first affecting the apex passes with equal intensity to all parts of the stalk, but one side (the upper or the under) in all likelihood is more sensitive than the other, a view which is supported by a comparison with the behaviour of tendrils. These organs behave purely *tropistically* after contact stimulation, after chemical and thermal stimuli, however, they behave *nastically*. The curvature which is induced by *heating* or *cooling* is, according to CORRENS (1896 a), always in the *same* direction; it is the under side always that exhibits concave curvature, beginning from the apex in the case of the tendril, and the result is the same whether the temperature of the tendril is, by *conduction*, altered to a similar extent on *all* sides, or whether it be heated on any *one* side by *radiation*. We must therefore conclude that heating of *any part* of the tendril results in a uniform rise of temperature and that the consequent movement is always the same, viz. that determined by the physiological dorsiventrality of the tendril. At the same time it is very remarkable that tendrils which react to contact equally well all round always curve so that only their under sides become concave when subjected to heat stimulus. [The mechanics of tendril curvature following on heating does not differ from that resulting from contact stimulus; the same is true of curvature induced by wounds, save that in that case the transmission of the stimulus is very rapid and far-reaching (FITTING, 1903 b).]

Drosera (and the tendril also) presents us with very interesting transitions between tropistic and nastic reactions. Following on mechanical and chemical stimulation, very delicate nastic movements, which are closely related to those of *Drosera*, occur also in other carnivorous plants, as for instance in *Dionaea muscipula*, *Aldrovanda vesiculosa* and, less markedly, in *Pinguicula*. Detailed research on these forms, comparable to those which have been made on *Drosera*, have not as yet been carried out, though much required, so that we had best content ourselves with this brief reference; still less have we space to discuss haptotropic curvatures in lower forms (Mucorinae, ERRERA, 1881; TRZEBINSKY, 1902; Algae, NORDHAUSEN, 1900).

Bibliography to Lecture XXXVIII.

BARANETZKY. 1901. Flora, 89, 138.
BATALIN. 1877. Flora, 60, 36.
CORRENS. 1896 a. Bot. Ztg. 54, 1.
CORRENS. 1896 b. Ibid. 54, 21.
DARWIN. 1876 a. Die Bewegungen u. Lebensweise kletternder Pflanzen (CARUS). Stuttgart.
DARWIN. 1876 b. Insektenfressende Pflanzen (CARUS). Stuttgart.
ERRERA. 1884. Bot. Ztg. 42, 497.
EWART. 1898. Annales Jard. bot. de Buitenzorg, 15, 187.
FITTING. 1902. Ber. d. bot. Gesell. 20, 373.
FITTING. 1903 a. Jahrb. f. wiss. Bot. 38, 545.
[FITTING. 1903 b. Ibid. 39, 424.]
HABERLANDT. 1901. Sinnesorgane i. Pflanzenreich z. Perzeption mechanischer Reize. Leipzig.
MACDOUGAL. 1896. Ber. d. bot. Gesell. 14, 151.
MÜLLER, O. 1887. Cohn's Beitr. z. Biol. 4, 97.
[NĚMEC. 1904. Beihf. bot. Centrbl. 17, 52.]
[NEWCOMBE. 1902. Beihf. bot. Centrbl. 12, 243.]

Nordhausen. 1900. Jahrb. f. wiss. Bot. 34, 235.
Peirce. 1894. Annals of Botany, 8, 53.
Pfeffer. 1885. Unters. bot. Inst. Tübingen, 1, 483.
Rosenberg. 1899. Phys.-cytolog. Unters. über *Drosera rotundifolia*. Upsala.
Sachs. 1873. Arb. bot. Instit. Würzburg, 1, 385.
Trzebinsky. 1902. Anzeiger der Akad. zu Krakau.
Treub. 1882–3. Annales Jard. bot. Buitenzorg, 3.
De Vries. 1886. Bot. Ztg. 44, 1.

LECTURE XXXIX

NYCTITROPISM

Many plant organs, especially foliage and floral leaves, take up, towards evening, positions other than those which they occupy by day. Petals and perianth leaves, for example, bend outwards by day so as to *open* the flower, and inwards at night so as to *close* it. Corresponding movements take place in entire inflorescences as, for example, in those of the Compositae ; the capitulum may be said to open when the ray flowers, or all the flowers, of the head curve outwardly, and to shut when they curve inwards towards the centre. Many foliage-leaves, also, may be said to exhibit opening and closing movements, not merely when they open and close in the bud but also when, arranged in pairs on an axis, they exhibit movements towards and away from each other. In other cases, speaking generally, we may employ the terms *night position* and *day position* for the closed and open conditions respectively ; thus, for example, the umbels of *Daucus* are directed vertically downwards in the evening and stand erect by day. The night position may also be described as the *sleep position*.

Day and night positions may arise by the combined action of geotropism and heliotropism. Thus Vöchting (1888) observed in the case of *Malva verticillata*, that the leaves, when illuminated *from below*, turned their laminae downwards during the day, but during the night became erect geotropically. The sleep movements in leaves and flowers referred to above cannot, however, be explained by assuming such a combination of heliotropism and geotropism, for, as a rule, they have nothing to do with tropisms at all, although they are frequently occasioned by light or, in other cases, by heat. Light and heat do *not* operate in these curvatures as they do in heliotropism and thermotropism proper, where opposite sides of the plant are subjected to stimuli of unequal intensity ; while in heliotropism and thermotropism we have to deal with a variable *regional* distribution of heat and light, in the present case we have to do with movements which are induced by these same factors operating at different *times*. In other words, the plant reacts to *variations* in the degree of *illumination* and *variations* in *temperature*, and its reactions are not *tropistic* in character but *nastic* ; we might, in fact, describe these opening and closing movements as thermonastic and photonastic respectively, and might, at the same time, characterize, with greater accuracy, the outward curvature as the result of epinasty, and the inward curvature as the result of hyponasty; that is to say, the movements are photoepinastic or photohyponastic. These same movements have also been described as nyctitropic and the phenomena as those of nyctitropism. Although this phrase has now come into common use, we must nevertheless point out that it is incorrect from two points of view; first, because we are not dealing in these instances with a *tropism* at all, and, secondly, because it is not darkness (*νύξ*) that is the releasing stimulus, but the *alternation* of light and darkness.

The reason why we associate nyctitropism with haptotropism is that the mechanics of nyctitropic movement may, in *many* cases, be compared with that exhibited by tendrils; the likeness between these two sets of phenomena is especially evident if we compare the thermonastic curvatures of tendrils with the nyctitropic movements which we find manifested by many flowers. The thermonastic curvatures of tendrils arise, as FITTING (1903) has established, exactly in the same way as do haptotropic curvatures. On cooling or heating a growth acceleration makes its appearance, which is most vigorous on the upper side near the periphery, but which spreads to the middle zone also, and fades away in the neighbourhood of the contracting under or concave side. Some time afterwards, when the temperature has again become constant, the tendril once more straightens itself as a result of an inverse growth process. Let us now compare this with what we see in a spring flower, such as a tulip or a crocus. When the temperature has risen sufficiently, vigorous growth takes place on the upper side of the perianth; this becomes convex, and the individual leaves bend more or less outwards, in accordance with the rise in temperature. Visible curvatures may be distinguished even after the application of feeble stimuli; in the crocus, for example, an elevation of temperature of one half a degree is, according to PFEFFER (1873), sufficient to cause a movement. In the tulip the greatest curvature takes place in the basal part of the perianth leaves, and we may convince ourselves of this fact by carefully measuring fixed distances of appropriate length on opposite sides of the perianth leaf. In the following table, measurements of this kind are recorded which aim at showing the effect of increase of temperature on the rate of opening of two flowers. The numbers represent *percentage increments per hour*. The flowers were kept at a temperature of 11° C. from 5.30 p.m. one evening until 12.40 p.m. the next day, when they were transferred to a temperature of 18° C. (JOST, 1898).

	Temperature 11° C.		Temperature 18° C.			
	% increase per hr. from		1st hr.	2nd hr.	3rd hr.	4th hr.
	5.30 p.m.–9 a.m.	9–12	12.40–1.40	1.40–2.40	2.40–3.40	3.40–4.40
Flower No. 1						
Outside . .	0·1	0·2	0·0	7·4	1·8	0·4
Inside . . .	0·3	0·4	6·8	0·5	3·4	0·1
Flower No. 2						
Outside . .	0·1	0·0	1·5	4·7	0·0	1·2
Inside . . .	0·3	0·1	3·9	0·0	0·6	0·3

If we consider the first flower, we find that at a constant temperature of 11° C. an extremely slight increase takes place in the course of several hours; but in the first hour after the higher temperature had made itself felt the inside grows rapidly, the outside not at all. In the second hour, the relations are entirely reversed; the outside grows rapidly, the inside scarcely at all. The second flower behaves in exactly the same manner. The curvature of the perianth leaf naturally goes hand-in-hand with this distribution of growth, that is to say, in the first hour the flower opens vigorously, and during the second hour as vigorously closes. This phenomenon corresponds in all respects to that exhibited by tendrils, for the stimulus results not in a movement in *one* direction only, but in a backward and forward oscillation. As in the case of tendrils, the time which is necessary for the carrying out of an oscillation varies greatly in different flowers. The backward movement follows very rapidly in the case of *Tulipa*, but it is not so in all cases; in *Crocus*, two or three hours elapse, and in other flowers an even longer period, before the backward movement begins. It can scarcely be doubted that we have here to do with a case of autotropism, and we may imagine that it is due, as is the curvature itself, to a growth acceleration in the *median zone* also. It is possible that between those two periods of more vigorous growth there intervenes a time when growth

is retarded or ceases entirely; measurements taken at shorter intervals than have as yet been made can alone settle this question. [Measurements of this kind have been made meanwhile by WIEDERSHEIM (1904) with results precisely of the nature one would expect.] The growth acceleration of the middle zone associated with the movements we are discussing is remarkably apparent in the measurements above quoted.

Hourly percentage of growth in the median zone.

	At 11° C.		After heating to 18° C.			
	5.30–9 p.m.	9–12 p.m.	1st hr.	2nd hr.	3rd hr.	4th hr
Flower No. 1	0·2	0·3	3·4	4·0	2·7	0·25
Flower No. 2	0·2	0·0	2·7	2·3	0·3	0·7

The acceleration shows itself most clearly if we compare growth in the first and second hours after warming with that exhibited in the fourth hour when exposed to continuous but uniformly higher temperature.

The effect of cooling may best be studied in the measurements recorded by PFEFFER (1875, p. 125). He subjected crocus flowers, which had been exposed for about twenty-four hours to a temperature of 17° to 18° C., to a temperature of 7° to 7½° C., and observed that the flowers *closed*. Increased growth set in on the outsides of the perianth leaves, which again affected the middle zone, but which after a short time became very considerably reduced. In support of this statement we give below certain of PFEFFER's measurements of the growth of the *middle zone* of the crocus in percentages per hour.

	At 17°–18° C.		At 7°–7½° C.		
	4 p.m.–9 a.m.	9 a.m.–12 noon	1st hf. hr.	2nd hf. hr.	Next 3 hrs.
Crocus No. 1	0·64	0·70	4·65	1·87	0·41
Crocus No. 2	0·67	0·74	6·21	3·27	0·34

It is unknown whether an opening movement, i.e. an autotropic reverse action, takes place at lower constant temperatures still, but it is very probable that that will be found to be the case.

The act of *changing* from one temperature to another increases the average growth of the perianth leaves much above that exhibited at *constant* temperatures; it affects the upper and under sides *differently*, however, and thus induces curvature. In all probability, a curvature may even make its appearance at such temperatures as lie beyond the maxima and minima of growth when they affect the plants constantly (comp. BURGERSTEIN, 1902). The analogies between these movements and those of tendrils, especially the analogies with *thermonastic* movements, are as we have already said, very great. They express themselves in this, that *during* the movements due to stimulation, both incurvature and recurvature, a new stimulus is always released owing to elevation of temperature; the plants do not become accustomed to the stimulus, or, at least, do so only gradually, for we can continue to induce opening movements with appropriate elevations of temperature for many hours (JOST, 1898). All the same we must not forget that there are *differences* to be taken account of. In the tendril the most vigorous growth acceleration *always* takes place on the *upper* side whether temperature be increased or decreased; in the floral-leaves, on the other hand, the *upper* side becomes convex when the temperature is *raised* and the *under* side when the temperature is *decreased*.

Not all flowers respond with nyctitropic movements as *Tulipa* and *Crocus* do, exclusively or especially, when the temperature is altered; some respond only to alterations in light, others exhibit nyctitropic movements only when light *and* temperature are altered at the same time. [As to other factors in nyctitropic movements compare HENSEL (1905).] Among plants which are sensitive to alterations in light we must place the Compositae, where shading

causes the closure of the capitulum, as cooling does in the flowers of *Crocus*, and illumination causes opening, as heating does in the case of *Crocus*. In nature, as a rule, increased light is accompanied by an elevation and decreased light by a reduction in temperature. Observations as yet available show that curvature either in the lower tubular or in the upper flattened portions of the corolla is effected by the same mechanical means as are *thermonastic* curvatures. There are various difficulties, due partly to the nature of the movements themselves and partly to the fact that insufficient measurements have been carried out in this direction, which render it impossible for us to enter into greater detail on these questions at the present moment.

Nyctitropic growth movements occur in the foliage-leaves of a very large number of plants of very distinct families, e.g. Alsineae, Compositae, Solanaceae, Balsamineae, &c. (BATALIN, 1873). As a rule, simple leaves take up a more or less horizontal position by day, while in the evening the laminae assume a vertical position, due either to a curvature in the petiole or at the base of the lamina. Thus the leaves either *droop* at night time, as in *Impatiens*, *Polygonum convolvulus*, *Sida napaea*, &c., or they stand *erect*, so as to press themselves against the bud, as in *Chenopodium*, *Brassica*, *Polygonum aviculare*, *Stellaria*, *Linum*, &c. That the factor concerned in these movements is an alteration in *light* may easily be proved in a large number of cases, for placing the leaves of *Impatiens* in darkness at midday induces a very marked drooping. More exact research is certainly required as to the sensitivity of such leaves for changes in temperature.

Certain conclusions as to the mechanics of curvature may be drawn from PFEFFER'S (1875) measurements, although a more exhaustive investigation of these is urgently needed, especially after FITTING'S work on tendrils. The following data are taken from PFEFFER'S observations :—

		Impatiens noli-me-tangere. Length of a section originally 100 units long.	
		Upper side.	Under side.
9.30 a.m.	Light	100	100
12.30 p.m.	Dark	100	100
1.30 p.m.	Dark	105	99·4
3·30 p.m.	Light	105	102·8

Although no growth could be observed in the leaf when uniformly illuminated for 3 hours before midday, when the leaf was suddenly darkened the upper side increased 5 per cent. in the course of one hour and the under side decreased ½ per cent.; in the course of 2 hours, after the leaves had been again exposed to light, the under side increased almost 3 per cent., while the upper side remained stationary. The reversion of the curvature, however, which is associated with this more vigorous growth of the under side may also take place *without* any illumination. It would thus appear as if in this case an oscillation to and fro might be brought about by mere darkening, each movement being accompanied by considerable growth acceleration. The close analogy with tendrils is in no sense supported by this result, since the movements of the leaf of *Impatiens* are, as we have seen, of a more complicated character. We shall take an opportunity later on of returning to this subject. These same complications render it impossible for us as yet to answer the question whether the influence of illumination is analogous to that of darkening or not. Comparing the behaviour of flowers when the temperature is raised, we are led to expect a temporary increase in growth when light is intensified ; the evidence in favour of this view is, however, by no means above criticism. [Evidence in favour of this conception has now been adduced by WIEDERSHEIM (1904).]

Nyctitropic movements in the foliage-leaves hitherto mentioned, are

carried out only whilst the leaf is still growing; the amplitude of the movements decreases proportionally with the age of the leaf. Other leaves, however, retain the power of nyctitropic movement even in the full-grown condition, but, as is to be expected, they are distinguished by the presence of articulations at their bases. As to the special capabilities of the articulation, we have already learned something when treating of heliotropism and geotropism. Most, but by no means all, leaves which are provided with articulations exhibit nyctitropic movements, such, for example, as numberless Leguminosae, many Oxalidaceae and Marantaceae, several Euphorbiaceae (*Phyllanthus*), Zygophyllaceae (*Porliera*), and Hydropterideae (*Marsilia*), and many others (HANSGIRG, 1893, p. 131). In most cases, not *one* only but several articulations, are concerned in the movement; in bipinnate leaves, for example, we find one pulvinus at the base of the main petiole, another at the base of each of the secondary petioles, and yet another at the base of each individual leaflet, and when all these pulvini operate at the same time the movement of the leaf which results must necessarily be complicated. In *Mimosa pudica*, for example, in the day position the chief petiole forms with the stem an angle of about 60° upwards; there are two pairs of secondary petioles present, the basal pair of which stands almost at right angles to the primary petiole, whilst the two apical ones form an angle of about 60° with each other forwards; finally, the leaflets spread out horizontally, and form angles of 90° with the secondary petioles in the same plane. The position of the leaf at night is entirely different, for the primary petiole bends downwards as much as 80° to 100°. The four secondary petioles bend forwards so as to place themselves almost parallel with the long axis of the chief petiole; the leaflets bend upwards and approximate in pairs, their upper sides touching. At the same time they suffer slight twisting, and now form an acute angle forwards, with the secondary petiole, the basal ones overlapping the apical ones like the tiles on a roof. The day and night positions are sketched at Fig. 159, in Lecture XL. In most cases it is only the articulations directly associated with the leaflets that exhibit these movements clearly; the leaflets are in *Mimosa* turned *upwards* at night, but in many other plants they are turned *downwards*. The behaviour of the leaflets in *Acacia*, in *Hypocrepis*, and also in *Coronilla* (Fig. 158, *III*) is perfectly similar to that of the leaflets in *Mimosa*, save that in the last case the leaflets are directed backwards instead of forwards. In *Trifolium* the terminal leaflet is simply raised up while the lateral leaflets are not only raised, but at the same time undergo a torsion of about 90°. A single downward curvature in the articulation occurs in *Robinia*, *Amicia*, *Phaseolus*, and in

Fig. 158. *I*, *Desmodium gyrans* in day position. *II*, The same in night position. *III*, *Coronilla rosea* in sleep position. After DARWIN, (1881).

Desmodium gyrans (Fig. 158, *I* and *II*); in the case of *Phyllanthus*, torsion occurs as well as curvature, so that in the night position the leaflets are folded together in pairs with their *upper surfaces* in contact with each other. Amidst all these variations, which the citation of further examples would only add to (HANSGIRG, 1893), one general fact comes out, namely, that in the night position the leaf surfaces are *vertical*.

It may be easily shown, say in the case of *Phaseolus* or *Acacia lophantha*, that darkening is the cause of the sleep position, and light the cause of the day position, but the reactions, for reasons which we shall learn later on, are not manifested equally well at all hours of the day; but if the plant be placed in the dark in the afternoon and illuminated in the forenoon we always obtain a very rapid result. Under all conditions, every movement of this kind is followed by a reverse movement later on.

As is to be expected, sensitivity to changes in *temperature* is also manifested by the articulations of motile leaves, although it cannot be demonstrated in all cases with equal ease. There is no difficulty in demonstrating movements resulting from changes in temperature in such leaves as those of *Mimosa*, *Acacia lophantha*, and *Phaseolus multiflorus*, which react well when light is excluded, and it may be easily shown (JOST, 1898) that an increase in temperature operates in the same way as an increase of light, and that cooling has the same effect as darkening. The case, however, is rendered more complicated in consequence of the fact that a rapid rise of temperature, dependent perhaps as well on the absolute height of the temperature, also brings about closure. The question now comes to be, whether this closure, due to a rapid rise of temperature, is really identical with the closure due to cooling or darkening, or whether it only resembles it superficially. That the latter must be the correct view would appear to be proved by the behaviour of plants like *Robinia* and *Phaseolus*, whose leaves, when subjected to a considerable rise in temperature, do not reach the sleep position, but go on rising until they succeed in orientating themselves in the same way as they do under the influence of too intense light. At p. 465 we have drawn special attention to this point in the case of *Robinia*, and described it as a heliotropic reaction, but we must not omit to mention here that frequently this 'profile' position occurring in *Robinia* in the open does not exhibit that relationship to light which, were it heliotropic, it must have; hence we conclude that the profile position of *Robinia* may perhaps be in many cases not a heliotropic reaction at all; it must, on the contrary, be quite independent of the *direction* of light, and may be caused only by too high a temperature or too intense light. The closure of many flowers may also be the result of too intense light, and to OLTMANNS (1895) we owe the demonstration of the fact that one and the same flower (e. g. of *Lactuca*), after being made to open by illumination, may after a time be made to close again by increasing the light intensity as well as by darkening. The condition brought about by too great an intensity of heat or light is also known as 'day-sleep'.

If we now inquire into the mechanical causes of nyctitropic movements in articulations we discover first of all that the curvature is effected without any growth taking place, since after having performed two opposite motions the articulation has not elongated (PFEFFER, 1875). We have here to deal, therefore, not with nutation movements but with variation movements; as a matter of fact, the expansive efforts of the convex side must be due to alterations in *turgidity*. In young pulvini undoubtedly growth also occurs. An elongation in one-half of the articulation owing to osmotic activity might arise just as well by increase of turgor pressure as by a decrease in the rigidity of the cell-wall. Omitting for the moment alterations in the elasticity of the membrane as being the less probable cause, curvature of the articulation might take place either (1) when the turgor pressure increases in the convex half of the

pulvinus, (2) when it decreases in the concave side, (3) when both these conditions occur, or (4) when an alteration in turgidity of the same kind but of unequal amount takes place in both halves of the pulvinus. We might imagine that it would not be difficult to determine which of these four possibilities is the correct one with the aid of the plasmolytic method; but HILBURG (1881) found it impossible to demonstrate any plasmolytic difference between darkened and illuminated articulations. This is all the more astonishing when we remember that in articulations which are geotropically stimulated, differences in the turgidity of the cells on opposite sides may readily be demonstrated by the plasmolytic method. The reason for the failure in the present instance is as yet by no means apparent, nevertheless we must assume that differences in turgidity are the causes of nyctitropic movement, and efforts must be made in some roundabout way to obtain definite information on the subject, as, for example, by determining the resistance to flexion and by experiments with articulations which have been operated upon.

As long ago as 1848, BRÜCKE showed that the resistance to flexion in the articulation was increased by darkening. In order to determine this resistance he simply used the statical moment of the leaf and carried out his experiment on the primary articulation of *Mimosa* in the following manner. He inclined the plant carefully until the petiole of the leaf under consideration occupied a horizontal position, that is, until the weight of the leaf exerted its maximum effect on the articulation. After measuring the angle (α) between the petiole and the stem, he turned the plant through an angle of 180° until the petiole was again horizontal and once more measured the angle (α'). The difference between these two angles ($\alpha - \alpha'$) affords an estimate of the resistance to flexion of the articulation, since as the resistance increases the value of the remainder decreases. In two experiments with *Mimosa*, BRÜCKE found this difference to be quite as great in the evening as in the morning or afternoon, but in two other experiments the difference was markedly less (namely 12° instead of 21°, and 15° instead of 27°). PFEFFER (1875) found that in *Phaseolus* the difference was 18° to 20° in daylight, 9° to 10° in the dark, and he was able to establish the fact that in many other plants an increase in the resistance took place in the evening. The increase in the resistance to flexion expresses nothing more, however, than that the tension of the parenchyma of the articulation against the central vascular bundle has increased; how this increase in tension is distributed in the articulation, whether all longitudinal areas participate or whether the osmotic swelling takes place only at definite situations, we do not at present know. It is even possible that turgor is reduced on one side only when it is *increased* on the opposite side.

Since no conclusion can be drawn as to the mechanical cause of the bending from the increase in the resistance to flexion, it will be necessary to experiment with half articulations after removal of the other halves. With this end in view PFEFFER removed the upper half of the articulation in the case of one primary leaf of *Phaseolus*, and the under half in another leaf, and arranged the leaves in an appropriate dynamometer so that the pressure activity of the expanding articulation could be recorded. The results of these two experiments were very remarkable; both halves of the articulation reacted in exactly the same way: when darkened expansion took place, when illuminated contraction, in each half. The movements which the leaf executed, when provided with one half-articulation only, were exactly opposite in direction according to whether the upper or the under half of the articulation was retained. The leaf provided with the upper half of an articulation became depressed upon darkening, and elevated itself when illuminated; the leaf provided with the under half of the articulation elevated itself in the dark and became depressed in light. Since, in the dark, both halves of the articulation show increased turgidity the conditions

would appear to coincide with the *fourth* possibility mentioned above, and a curving is only possible if the osmotic swelling on the convex side is greater, or reaches its maximum value *more rapidly* than it does on the concave side. This, as a matter of fact, was what PFEFFER assumed, and he held that nyctitropic curvature resulted from changes in the two half-articulations, *similar* in character but of *unequal rapidity*. As the turgidity on the concave side gradually increases the conditions become favourable for that reverse curvature which, as we have remarked, always takes place.

This conception cannot, however, be held to be *directly* proved, since PFEFFER'S results, obtained from experiments with half-articulations can by no means be universally confirmed; on the contrary, certain experiments have been made (SCHWENDENER, 1898; JOST, 1898) on the behaviour of half-articulations which gave results entirely contradictory of PFEFFER'S observations; when darkened, the upper half-articulation *expanded* whilst the under one *contracted*. This would tend to show that the alterations in the degree of illumination influenced the expansive efforts of the two halves in exactly opposite ways; the reverse curvature would not then be an immediate result of an external stimulus as in PFEFFER'S theory, but, as in tendrils and in nyctitropic growth movements, would, in the first instance, be a *result of the reaction* arising from autotropism. [WIEDERSHEIM (1904) is inclined to refer the dissimilar results of the removal of half-articulations to differences in the mode of carrying out the experiments. He maintains that his own special observations support PFEFFER'S theory, but we are not inclined to agree with him in that conclusion.]

We must content ourselves at present with pointing out that a completely satisfactory theory of nyctitropic pulvinus movements is not as yet forthcoming; such a theory can only be established after new and exhaustive experimental research. For reasons which we will appreciate later, it will be necessary first of all to study nyctitropic movement in leaves which have been previously cultivated under *perfectly constant* external conditions, more especially where there are no variations in light and temperature. Inquiry must then be made whether complete extirpation of one half-articulation really moderates the reaction of the other half in its original form, or whether it influences it correlatively. Further, it is necessary to prove whether the cell-membranes remain entirely unaltered so far as their elasticity is concerned, and whether the alterations in expansion are due *only* to alterations of osmotic pressure. The increase in the resistance to flexion of the entire articulation in the evening has not yet been established beyond doubt, for SCHWENDENER (1897) has recently been unable to confirm it, and it did not always make its appearance in BRÜCKE'S experiments (compare p. 506).

Should further research actually establish the fact that the alterations in the resistance to flexion of the articulation either do not occur regularly or have not the significance which we have ascribed to them, then the separation of *genuine* nyctitropic movement from so-called day-sleep movement cannot be carried out. In the case of *Oxalis*, PFEFFER (1876) found that the day-sleep position in direct sunlight went hand in hand with a *decrease* in the resistance to flexion of the articulation; this decrease must have arisen from a relaxation in *both* halves of the articulation, and especially in a *greater* relaxation in the concave half. Whether this relaxation is a phenomenon of *general* occurrence in day-sleep and whether it has essential significance in this relation must be left to further research to determine. At the same time, in all such experiments efforts must be made to discriminate sleep from the heliotropic profile position more accurately than is as yet possible; at the same time it cannot be denied that many profile positions are conditioned by a combination of heliotropism and nyctitropism.

While we regard such a combination of heliotropic and nyctitropic move-

ments as possible, we must, at the same time, consider the possible effect of *geotropism* on sleep movements. In fact, when we remember that a leaf when executing sleep movements comes into entirely different relations with gravity, the question arises whether gravity does not operate so as to bring it back again into the old position. *This* effect of gravity, however, has not as yet been demonstrated, indeed the effect is quite otherwise. In 1875, PFEFFER found that when a bean was placed in an inverted position, the sleep movements took place in the reverse direction so far as the *plant* was concerned; that is to say, they retained their relation to the direction of *gravity*. FISCHER (1890) showed that nyctitropic movements in the bean ceased when the plant was rotated on a klinostat. From these observations we may conclude that we have to deal in this case not with *nyctitropic*, but with *geotropic* movements, and that the plant in consequence of being darkened, reacts geotropically otherwise than before. Similar alterations in the geotropic rest position we found took place when rhizomes and roots were illuminated; but plants do not all behave in the same manner. Whilst *Lupinus albus* agrees in all essential features with *Phaseolus*, FISCHER found that in the case of *Amicia*, *Desmodium*, *Acacia*, *Mimosa*, &c., even after twelve days' rotation on the klinostat, nyctitropic movements retained completely their original direction. From this we must conclude that there are two types of plants which, following FISCHER, may be distinguished us autonyctitropic and geonyctitropic. We confine ourselves merely to the statement of this fact, but we may at least note that FISCHER's experiments are not so easy of explanation as one might at first sight think. NOLL (1892) has drawn attention to the possibility that dorsiventral organs are geotropically stimulated when rotated on a klinostat, and SCHWENDENER (1892) has shown that *Phaseolus*, at least in the first days during which the plant was rotated, preserved its sensitivity to changes in illumination. Further research in this subject will be necessary before we can give a clear account of the phenomena.

A very large number of, though by no means all, plant organs which carry out nyctitropic movements present difficulties in the explanation of the mechanics of the process of curvature for one special reason; the nyctitropic movements *continue* after the *cause*, namely alteration in illumination, *ceases* (*periodic* movements). This phenomenon may be remarkably well seen in *Mimosa* or *Acacia lophantha*, when these plants are kept in the dark at a constant temperature. For days they open their leaflets in the morning and close them in the evening at approximately the same time as those plants which are exposed to periodic alterations in illumination. These movements, which are of the nature of after-effects, gradually cease, since the leaf becomes pathological when kept in continued darkness, and changes are set up in it which lead, in the first place, to abolition of the power of movement, that is to say, to darkness-rigor (SACHS, 1863), and in the long run even to death. The absence of light induces this condition of rigor only indirectly, for we can render the leaves of *Mimosa* incapable of movement when completely illuminated also, if we at the same time deprive them of access to carbon-dioxide (VÖCHTING, 1891). On the other hand, leaves which have been cultivated in the dark live and exhibit movements for a *much longer* time in absence of light than those which have grown to maturity in light. Obviously the rigor is due to injuries suffered by the leaf in consequence of an interference with the chlorophyll function.

As we have mentioned above, these after-effects are also met with in leaves which carry out nyctitropic curvatures by growth, such as the leaves of *Nicotiana*; they are also met with in many flowers. On the other hand, they occur in leaves which, after being in the dark for a short time, cease to move, although they still retain their *capacity* for movement, as, for example, *Tulipa*, *Robinia pseudacacia*, or which carry out movements in the dark which are quite irregular and which bear no relationship to variations in light. These latter movements, the so-called autonomous movements, we will treat of in Lecture XLI.

The *periodicity* in the after-effects we have referred to are of extreme interest from several points of view; at the very commencement, however, it must be evident that the studies we have hitherto made on the subject of simple nyctitropic movement are incomplete in one important respect. We are unable in the individual case to distinguish what is the *direct consequence of a solitary stimulus* and what is an *after-effect*. Since the sleep position is brought about more rapidly by placing the plant in the dark in the evening than in the morning we may look on that as an instance of after-effect, and if the sleep movement is followed after a short time by an opening movement when the plant is placed in the dark in the morning, we must not regard this reverse movement in any sense as due to autotropism, but as an after-effect also. Just because after-effects frequently manifest themselves in nature we ventured to suggest above that nyctitropic movements should be studied in such plants as have been cultivated under constant external conditions; but this suggestion has not been as yet realized. On the other hand, many authors, and more especially PFEFFER, have shown that in certain plants which exhibit no autonomous movements, after-effects gradually cease when the plants are exposed to *continuous light*. Thus PFEFFER (1875) noticed that the periodic movements in *Acacia lophantha* and in *Impatiens*, when exposed to continuous light, became gradually weaker, until finally a *permanent day position* was reached. This result was to be expected, for SCHÜBLER (1873) had already pointed out that in the case of plants growing in Northern Norway the periodic movements they exhibited in midsummer disappeared for a considerable time and reappeared again after the commencement of the arctic night. The cessation of periodic movements as a result of exposure to continuous darkness, presents great difficulties, as we have already pointed out (p. 508). We do not as yet know whether leaves which become normal in complete darkness (JOST, 1895) assume a fixed night position when the external conditions are quite constant; the great majority of leaves, however, assume an abnormal rest position in darkness, because either their upper sides or their under sides exhibit increased growth (epinasty, hyponasty) (VINES, 1889).

The cessation of the periodic movements is especially of interest because it shows that these movements are not hereditarily characteristic of the plant, as one might at first imagine from their continuance in darkness. Other experiments lead to similar results, for we may by illuminating plants during the night and darkening them during the day shift the periodicity of movement as much as twelve hours. Periodic movements are, moreover, not developed once and for all; on the contrary, they apparently arise *gradually* in the course of the development of the plant. In order to study the question of the mode of *origin* of these movements we must at present confine ourselves exclusively to plants which have, when continuously illuminated, lost the power of performing periodic movements, since as yet no leaves have been cultivated under conditions which prohibited the *original* development of such movements. After the loss of periodic movement these leaves are in no sense in a state of rigor. In one experiment of PFEFFER'S (1875), a plant of *Acacia lophantha* closed its leaflets as soon as it was darkened, but after a few hours began to open them once more, and in less than 12 hours the leaflets were again almost completely extended. When kept in the dark continuously, they exhibited on the two following days, two oscillations, whose turning points were 18 to 24 hours apart. PFEFFER concluded from these and similar experiments that a solitary nyctitropic stimulus induced not one but a whole *series of oscillations*. If that be so, then the periodic movement must be brought about in such a way that the *solitary stimulus is combined with an after-effect*. This, however, is only possible if these secondary oscillations occur in the same rhythm as that in which the stimulus affects the plant in nature, that is to say, the periods between opening and closing must be about twelve hours in length. In the experiment with *Acacia* just described the oscillation was too

rapid on the first day and too slow on the second. If, however, after-effect and renewed stimulus do not coincide, an after-effect, as constant as we have seen exhibited in the dark by *Mimosa*, cannot arise. There are two possibilities by way of explanation of this contradiction; either regular and long continued periodic movement in the dark is not an after-effect at all but is, at least in part, a result of small oscillations in *temperature* which were not sufficiently carefully guarded against in many of the experiments hitherto performed, or the *oscillations* observed subsequent to a single darkening in PFEFFER'S experiments, in plants which had ceased to move in light, were not the result of this solitary stimulus but were the after-effects of a *previous* daily periodicity, which they had not yet quite lost, but which were only obscured in light and which had lost their regularity. It would be very remarkable, however, if a single darkening induced a maximum amplitude of movement, while in the case of plants which had lost their power of movement, e. g. *Siegesbeckia*, a new stimulus took five days to induce the maximum depression of the leaf.

Seeing that objections of this kind may be advanced against PFEFFER'S argument, it is necessary that experiments should be carried out on plants which have never shown periodic movements. They will, we have no doubt, lead to the result which PFEFFER has already indicated, namely, that periodic movements arise from a summation of individual stimuli and after-effect, the single stimulus inducing *several* oscillations which must be carried out with ever-decreasing amplitude. These oscillations following upon a single stimulus offer a subject for detailed investigation. First of all they must be definitely proved (for SCHWENDENER (1897–8) has called these facts in question, and JOST (1898) and PANTANELLI (1900) have failed to find them in *Robinia*), and it must also be shown how far the oscillation period depends upon the duration of the stimulus. It is quite possible that we might succeed in inducing periodic movements of six hours' duration or even less, but it is not outside the range of probability that the duration of the oscillation renders periods so short as these in the after-effects impossible. [As a matter of fact, SEMON (1905) has succeeded in inducing a 12-hour periodicity in the after-effect where, before illumination, the rhythm was 6-hourly or 24-hourly. All this goes to show how little indeed we do know even now about periodic movements.]

There are still many other complicated phenomena which militate against a thorough explanation of periodic movement; e. g., let us look at the case of flowers and at the tulip in particular. First of all when the flower is heated a single opening and closing movement only can be recognized; no *periodic* movements at all can be observed in the tulip. If these be possible we should perhaps obtain them most speedily by alternately heating and cooling the plant every hour; but perhaps they are absent altogether. In other flowers, however, such movements exhibit themselves markedly. OLTMANNS (1895) draws attention to many points which have as yet been but little cleared up. He finds that *Bellis perennis* opens its inflorescence after being 48 hours in the dark, whilst in the case of *Tragopogon* 8 to 12 hours is sufficient. He certainly regarded these opening movements not as after-effects of a previous periodicity, but looked upon *darkening* itself as the stimulus which induced opening, acting especially vigorously on night-flowering plants. A discussion of this and other results arrived at by OLTMANNS would lead us, however, too far; we may merely note that all these movements have not as yet been studied in a sufficiently experimental way, and that before we can arrive at an explanation of *periodicity* we must determine what part duration and intensity of light and of heat in the single stimulus play in the process. That the origin of periodic movements from a summation of the individual stimuli of light and darkness together with their after-effects is not quite so simple a matter as we might suppose, may be deduced from the behaviour of the chief petiole of *Mimosa*. After a single darkening it *raises*

itself up, as SCHWENDENER (1897) has already shown, but in the periodic movements it *droops* in the evening. This contradiction PFEFFER naturally did not fail to notice, and he has also attempted to explain it. He assumed that the drooping arose from a marked increase in the statical moment of the leaf which was necessarily associated with the forward movement of the secondary petioles described above, and he found that his supposition was confirmed by experiment. When movement in the secondary petioles was rendered mechanically impossible the evening drooping gradually ceased, but came back again when the secondary petioles were allowed freedom of movement. In spite of this ingenious experiment we have certain criticisms to advance against the correctness of the explanation. In nature the erection of the petiole takes place during the night and it may amount to from 40° to 80° in 6 to 8 hours. SCHILLING (1895) has, however, noticed that the compression of the articulation and sinking of the petiole (the statical moment of which had been greatly increased by hanging a weight upon it) was compensated in 10 to 15 *minutes*; and it is not obvious why the plant should require several *hours* to compensate evening drooping, if this drooping be due to purely mechanical causes. SCHWENDENER (1897) has advanced additional arguments against PFEFFER'S explanation, so that we may regard the sinking and rising of the petiole of *Mimosa* as a phenomenon of periodic movement not yet explained, and possibly as a phenomenon which has some special biological function to fulfil.

We cannot enter into a discussion of the mechanics of periodic movement; we may only notice that, according to PFEFFER, in nutation movements as in variation movements the *opposite* sides behave in *contrary* ways during the *after-effect*; this is especially well seen in the articulation because the *resistance to flexion of the entire articulation remains unaltered* owing to the expansion of the convex side and the *simultaneous* contraction of the concave side.

In conclusion we may ask ourselves what is the biological significance of nyctitropic movements; for that they have nothing to do with the 'sleep' of animals goes without saying. We must keep distinct in our minds the movements of foliage-leaves and those of floral-leaves. The position which foliage-leaves take up in the evening is essentially *vertical*; it is a matter of less moment manifestly, whether they bend upwards or bend downwards. The question is what is the advantage of this nocturnal vertical position to them? DARWIN (1881) has drawn attention to the fact that the leaf in the vertical position is less subject to heat radiation during the night than if it were in the horizontal position. But this radiation as such could injure the leaf only during cold nights, and yet at low temperatures nyctitropic movements cease; on the other hand, they occur during warm summer nights and are especially evident in tropical plants where injury due to cooling is out of the question. Hence STAHL (1897) has sought to explain the night position by considering it as a means of preventing the deposit of dew, and he has supported this idea, as far as it was possible to do so, by means of experiments. He looks upon the deposition of dew as injurious to the plant inasmuch as it prevents transpiration so long as the leaves are wet.

As to the biological significance of nyctitropic movement in flowers little trustworthy is as yet known. It is scarcely possible that we have to do here with any transpiration phenomena, as in the case of foliage-leaves; in all probability quite different explanations must be given of the movement of floral-leaves in different groups of plants. We may imagine that in the case of spring flowers which are especially sensitive to heat stimulus, the closing of the flowers is a protection against cold. Flowers which open in the evening are obviously adapted to the visits of those insects which effect pollination and which are nocturnal in habit; insects which are active during the day would thus be entirely excluded from such flowers. Other types again which show opening and closing movements may thereby attain benefits with which we are as yet entirely unacquainted.

Bibliography to Lecture XXXIX.

BATALIN. 1873. Flora, 56, 450.
BRÜCKE. 1848. Archiv f. Anat. u. Physiol. Ostwald's Klassiker, No. 95.
BURGERSTEIN. 1902. Bewegungsersch. d. Perigonblätter von *Tulipa* u. *Crocus* (Jahresbericht Erzherzog Rainer Gymnasium. Vienna).
DARWIN. 1881. Das Bewegungsvermögen d. Pflanzen (CARUS.) Stuttgart.
FISCHER, ALFR. 1890. Bot. Ztg. 48, 673.
FITTING. 1903. Jahrb. f. wiss. Bot. 38, 545 [and 39, 424].
HANSGIRG. 1893. Physiologische und phycophytologische Untersuchungen. Prag.
[HENSEL. 1905. Nebraska University Studies. V. No. 3.]
HILBURG. 1881. Unters. bot. Inst. Tübingen, 1, 23.
JOST. 1895. Jahrb. f. wiss. Bot. 27, 403.
JOST. 1898. Ibid. 31, 345.
NOLL. 1892. Heterogene Induktion. Leipzig.
OLTMANNS. 1895. Bot. Ztg. 53, 31.
PANTANELLI. 1900. Atti soc. dei Naturalisti. IV, 2. Modena.
PFEFFER. 1873. Physiologische Untersuchungen. Leipzig.
PFEFFER. 1875. Periodische Bewegungen. Leipzig.
SACHS. 1863. Flora, 46, 449.
SCHILLING. 1895. Einfl. von Bewegungshemmungen auf Arbeitsleistung. Jena.
SCHWENDENER. 1897. Sitzungsber. Berliner Akad. 228.
SCHWENDENER. 1898. Ibid. 176.
SCHWENDENER and KRABBE. 1892. Abh. Berl. Akad.
SCHÜBLER. 1873. Die Pflanzenwelt Norwegens. Christiania.
[SEMON. 1905. Biol. Centrbl. 25, 241.]
STAHL. 1897. Bot. Ztg. 55, 71.
VINES. 1889. Annals of Bot. 3.
VÖCHTING. 1888. Bot. Ztg. 46, 501.
VÖCHTING. 1891. Ibid. 49, 113.
[WIEDERSHEIM. 1904. Jahrb. f. wiss. Bot. 40, 230.]

LECTURE XL

MOVEMENTS RESULTING FROM SHOCK

IN the last lecture, in which we treated of nyctitropic movements, we had frequent occasion to mention *Mimosa*, but we must not lose sight of the fact that this plant is sensitive to *other* stimuli as well as to alterations of light and temperature. In fact *Mimosa* exhibits another sensitivity much more prominently than it does nyctitropism ; a gentle shaking of the leaf is sufficient to induce at once a very characteristic change in position. This new rest position corresponds exactly with the sleep position, for the primary petiole droops considerably and the secondary petioles move forwards almost parallel with the long axis of the primary petiole, the leaflets folding together in pairs and, at the same time, bending obliquely forwards. In spite of their great superficial similarity the two movements are in reality quite distinct and arise in quite different ways. The reaction induced by shock differs further from the sleep movement in the rapidity with which it is carried out ; a few seconds are sufficient for its execution. On account of the rapidity with which the response takes place, the movement of the *Mimosa* leaf is one of those with which we have been longest acquainted in plant life, a movement which, owing to its rapidity alone, has been compared with the responses to stimuli exhibited by animals, and which was always looked upon as a genuine reflex action even at a time when the absence of sensitivity in plants was regarded as one of the distinctions between plants and animals. These movements in the 'Sensitive plant', in addition to geotropic movements,

have figured prominently in the history of that department of physiology which deals with sensitivity.

Apart from general habit, we may compare the stimulus-movements just discussed in *Mimosa*, but which are manifested by other plants as well, with nyctitropic movement, inasmuch as we have to deal in this case with the assumption of a *new position of equilibrium* as a result of stimulus, which, however, is *temporary* only; for after a short interval we find that the stimulated leaf regains its normal day position, and that too without being again stimulated, just as the darkened leaf re-erects itself of its own accord in daylight. Further, just as the day position may be again assumed in continued darkness, so also a leaf of *Mimosa* may once more attain the normal day position after continuous small shocks rapidly applied. There are other and fundamental differences, however, between response to shock and to changes in illumination. As already mentioned, there is a difference both in the rapidity as well as in the mechanics of the movement in the two cases; to both of these points we shall

Fig. 159. *Mimosa pudica.* On the left an unstimulated twig in the day position; on the right the same after having been shaken. Reduced. (From the Bonn Textbook.)

recur presently. A further point of distinction lies in the fact that the shock stimulus under natural conditions is not periodic and that no after-affects have as yet been determined. In all probability, even if the shock stimulus be applied for a long time at regular intervals, no oscillations would manifest themselves after the cessation of the stimulation. The movements at present under consideration differ from those of nyctitropism in yet other respects, viz. in their significance in the plant economy, and, in connexion with that, in the nature of the releasing stimulus, and finally in their occurrence. The releasing stimulus may be not merely a *shock*, but also the mechanical and *chemical* influence of different bodies and the biological significance of movements due to shock is apparently quite different in different plants. In foliage-leaves, such as those of *Mimosa*, the movement is apparently intended to frighten away any animal that is calculated to injure the plant. There are special difficulties in proving the exact truth of this theory in this special instance, because it is only when it immediately precedes the visit of the animal that the movement can make any impression on the visitor; larger animals will be puzzled by the altered

appearance of the plants and smaller ones will be frightened by the movement itself. In the great majority of cases, however, where leaves react in this way to a shock stimulus, the response, even after a vigorous excitation, appears very slowly. The object of the movements in stamens and stigmas is much more apparent, for in these cases they are obviously almost always related to the mode of pollination of the flower.

Let us now study the movement in *Mimosa* a little more closely. As already stated curvatures take place in all three sets of articulations. In order to study the changes which precede these movements we will naturally confine ourselves to the largest pulvinus at the base of the primary petiole. Curvature in this pulvinus occurs not only when the whole plant is shaken but also when the articulation itself is subjected to a slight shock. It may be easily shown that it is only the under half of the pulvinus that is sensitive, for even a gentle friction of that side with a piece of stick at once induces a response, while the upper side may be much more vigorously stimulated without any result ensuing. Since at last a response is obtained by vigorous stimulation of the upper side, the reason for this lies in the fact that the stimulus has been transmitted to the lower half of the cushion. Experimental evidence on this point was first brought forward by LINDSAY in 1790, who showed that when the upper half of the pulvinus was removed the leaf remained capable of carrying out these movements, but that rigor set in when the lower half was cut off. (For the older literature see PFEFFER, 1873 a).

The more exact conditions of curvature are to be obtained by investigating the resistance to flexion, and by determining the alterations in volume taking place in the two halves of the pulvinus. The resistance to flexion increases in the night position but decreases markedly after a shock stimulus. BRÜCKE (1848) found the *difference in the angles* (α–α') in the two positions (compare p. 506) after stimulation to be two or three times as great as in the unstimulated condition, and so was able to establish a fundamental difference between the similar 'sleep' and 'stimulated' positions of *Mimosa*. It is to PFEFFER (1873 a) that we owe accurate investigations as to the changes in volume of the articulation. He proved by measurements, taken with the aid of the microscope, that the upper half of the articulation showed a quite insignificant increase during the curvature, but that the under half *markedly decreased*. Since the movement was carried out just as well when the epidermis had been carefully removed, and since the axial vascular bundle need not, on account of its rigidity, be taken into account, the reduction in volume observed can be accounted for only by a contraction in the parenchyma on the under side. This contraction is accompanied by a relaxation in the two halves of the pulvinus, which may be directly estimated if the effort to droop on the part of the leaf be prevented by some opposing force. In a more recent research PFEFFER has estimated this pressure at between $2\frac{1}{2}$ and 5 atmospheres. The expansive powers of the under half of the pulvinus is reduced to this extent, while apparently no change of any kind occurs in the upper half.

Whence this decrease of expansive force on the under side of the pulvinus arises cannot be settled straight away. In order to answer this question clearly experiments on amputated leaves are essential. Such leaves exhibit rigor at once on amputation, but, if they be kept in a saturated atmosphere, they regain their capacity of response to stimulus after a time, the primary articulation exhibiting inward curvature after contact, which may have a radius of 5 mm., while normal articulations exhibit a curve with a radius of only 3–4 mm. After stimulation of the amputated leaf it is possible to see a certain extravasation of liquid from the cut surface, which, if evaporation be prevented, is again absorbed as the curvature becomes gradually relaxed. This liquid is excreted from the parenchyma of the sensitive half of the pulvinus, and it is only after vigorous stimulation that the upper half helps in producing the excretion, and

that tardily and feebly. There can be no doubt that it is the sensitive cells of the under side that extrude this liquid, which then finds its way into the intercellular spaces and from them to the cut surface.

As the air in the intercellular spaces becomes compressed by the excreted liquid, another phenomenon makes its appearance which was first observed by LINDSAY and, later, correctly interpreted by BRÜCKE. At the moment of stimulation the under side of the pulvinus assumes a darker colour, such as follows upon injection of water under the air-pump. This dark coloration takes place even although the articulation be mechanically prevented from curving (PFEFFER, 1873 a), and it follows that the change in colour is not caused by the contraction of the pulvinus and consequent approximation of the chloroplasts. The sudden reduction in the expansive power of the lower half of the pulvinus is obviously associated with an alteration in its turgidity, and hence arise two possible explanations of the phenomenon (compare PFEFFER, 1890, 326); either the elasticity of the membrane increases, or the pressure in the interior diminishes; either phenomenon would result in the excretion of water from the cells. Since, if there be no alteration in the pressure of the cell contents, the size of the cell depends on the elasticity of the cell-wall, obviously a contraction of the cell might be produced by a decrease in the elasticity of the membrane. This possibility is not precluded in the case of *Mimosa*, but since it is certainly not applicable to the very similar movements exhibited by the stamens of the Cynareae and, at the same time, is not very probable in itself, we need not consider it further. When the pressure in the cell decreases we naturally assume this to be due to a decreasing *osmotic* pressure, a decrease which may well amount to $2\frac{1}{2}$ to 5 atmospheres, and may be due either to the transformation of osmotically active substances into bodies with larger molecules, or to alterations in the permeability of the plasma, and an excretion of materials from the cell. As evidence of the *excretion of material* we may quote the fact that PFEFFER (1873 a) observed crystals of unknown nature appearing on evaporation of the liquid expressed from the intercellular spaces. Still there are several reasons for doubting this conclusion. It is a remarkable fact that plasmolytic research (HILBURG, 1881) affords no evidence of any decrease in osmotic pressure (compare p. 506). Further it is not very probable that the original turgor relationships would be at once reinstated by cutting the articulation.

As PFEFFER has suggested, it is possible that a decrease of the pressure as affecting the membrane may also be induced by alterations in the protoplasm, more especially alterations in its capacity for swelling.

No complete insight into the mechanism of the stimulus movement in *Mimosa* has as yet been obtained, although one thing is certain, viz., that there is a decrease in expansive power on the under side of the articulation. Expansion on the upper side arises only from the removal of the opposing pressure below; at the same time the weight of the leaf helps to squeeze the under side. Again, when the plant is placed horizontally or inverted, that is, when the weight of the leaf is rendered non-effective, contraction of the sensitive half of the pulvinus in response to stimulus still takes place, showing that the weight of the leaf is not necessary for performance of the movement.

The articulations at the bases of the secondary petioles and of the leaflets behave, so far as we know, in a precisely similar way to that just described for the pulvinus of the chief petiole.

The depression of the chief petiole takes place, as has been already pointed out, with considerable rapidity. BERT (1870) found that, in a plant laid horizontally, the movement was completed in 4–7 seconds, and that it was more rapid still when the weight of the leaf was allowed to operate at the same time. As soon as the petiole had reached the position of maximum depression it began again to raise itself, and in the course of 10–15 minutes the original position was again attained, so that the leaf became once more fully capable of responding

to stimuli, repeated at $\frac{1}{4}$ to $\frac{1}{2}$-hourly intervals. No investigations appear to have been made as to whether or not the extent of the movement remains unaltered according to the length of time the stimulation is continued. Before the normal position of the leaf is reached, however, about 5 minutes after the first stimulation, the leaf may again react to a new stimulus, but then the amplitude of the movement is less. It may be supposed that in this case the *capacity for reaction* is still limited, because the original expansive force in the under half of the pulvinus has not yet been recovered; on the other hand, the *sensitivity* may still be normal. Conversely the *sensitivity* is obviously inhibited by continuous, feeble vibrations, while the *capacity for response* might be restored. We may conclude from this that the leaf returns to its normal position *during the shaking* and once more regains its resistance to flexion; although the leaf was not sensitive to shaking continued for 2–3 hours, it became once more fully sensitive in 5–15 minutes after the cessation of the stimuli (PFEFFER, 1873 a). From this point of view there is a great difference between the present case and hapto- and nycti-tropism, where the organs do not become accustomed to stimulation or do so very slowly.

There are many allied problems in this subject which yet await solution. We know only that the sensitivity is in many respects dependent on external conditions. High temperature, bright light and excessive moisture render the plants extraordinarily sensitive, and under such conditions feeble stimuli operate as well as strong ones, the responsive movement reaching its *maximum*; but when the sensitivity of *Mimosa* is decreased, owing to low temperature, drought or the application of chloroform, ether, &c., feeble shocks induce a much more limited depression of the leaf than strong shocks. Although we have no detailed information on the dependence of the extent of the reaction on the amount of the stimulus, we are better acquainted with the nature of the stimuli to which *Mimosa* especially will respond. We have already found that feeble contact with the sensitive part of the articulation induces a response; it has yet to be determined, however, whether the immediate conditions correspond to those established for tendrils.

The fact that the reaction is induced just as well by a liquid, e.g., raindrops, as by solid bodies, shows in the plainest possible way the great difference in character that exists between sensitivity as exhibited by *Mimosa* and by tendrils. Both react to a shock *only*, for statical pressure is followed by no response in *Mimosa*. PFEFFER, for example, obtained *no* movement when he gradually increased the weight bearing on the sensitive part of the pulvinus up to 30 g. *Mimosa* reacts, however, to *every* shock, if it be sufficiently intense, and a *solitary* stimulus is generally enough to bring about a maximum reaction. In the case of tendrils, however, as we have seen, such shocks are operative only when they affect neighbouring parts with unequal intensity; in their case also, *several* simultaneous or consecutive shocks are always necessary whose individual effects are capable of summation. In *Mimosa*, on the other hand, every sudden deformation of the cells of the sensitive half of the pulvinus is perceived and is responded to by a movement. Special hairs, which are, however, *not confined* in their distribution to the sensitive pulvinus only, serve as additional organs of perception; when these hairs, formed of thick-walled cells, become bent as a result of pressure or a blow, the deformation of these cells must, owing to their varied individual connexion with the sensitive parenchyma, be greater than that due to an equally strong pressure applied to the outer surface of the pulvinus (HABERLANDT, 1901).

The leaves of *Mimosa* respond not only to a blow but also to injuries, and movements take place after an incision or after the action of a burning-glass, though dissimilar in intensity to those which are consequent on a blow. The stimulus effected by a wound is much more rapidly transmitted than that induced by a blow, but the execution of the movement from a mechanical point of view

appears to be identical in both cases. *Mimosa* is also sensitive to *chemical* stimuli (CORRENS, 1892). Many substances which induce movement, such as hydrochloric acid vapour, may certainly injure the plant so much as to cause death ; but ammonia may be made to induce movements without at the same time causing injury, provided the doses be carefully regulated, and repeated responses may be obtained by successive applications of the stimulant. Electric currents will also induce movements (BERT, 1870), and it is possible that the reactions which are consequent on high temperature and intense illumination (p. 505) are to be regarded rather as related to those resulting from a blow than to those which are induced by change in light intensity. Detailed investigations on this point have still to be undertaken, however.

The sensitivity above described is not confined to *Mimosa* ; other Leguminosae, such as *Neptunia oleracea* and *Desmanthus plenus*, and some Oxalidaceae, such as *Biophytum sensitivum*, are known to be very sensitive. To a more limited extent all Leguminosae and Oxalidaceae, possibly all plants with pulvinate leaves, are to be considered as sensitive to shock (HANSGIRG, 1893), only in these cases the movements are induced by more powerful stimuli and take place only under optimal external conditions. *One* blow is frequently not sufficient to bring about a visible response, although several blows, by summation of stimuli, gradually induce a movement (*Robinia*, *Oxalis* sp.). The sensitivity of these plants recalls in its character that of tendrils ; sensitivity to contact is closely related to sensitivity to shock, for between *Mimosa* and tendrils, as the two extremes of the series, there are many intermediate conditions. Into the discussion of these, however, we need not enter.

We have yet to consider the *transmission* of the stimulus, which has been most thoroughly studied in *Mimosa* and *Biophytum*. Let us look at the phenomenon as it manifests itself in *Mimosa*. When this plant is grown under suitable external conditions, not only does the leaf droop when friction is applied to the primary articulation, but after a brief interval the pinnae also assume the folded rest position. If, on the other hand, one of the outermost leaflets be stimulated, not only does that leaflet move but the movement spreads to the opposite leaflet and onwards to the pinnae inserted lower down the petiole, all of which fold together in pairs. It has already been noted that a wound operates much more effectively than mere contact. If the terminal pinnae be scorched with a glowing splinter or with a burning-glass, the stimulus is rapidly conveyed to the base of the secondary petiole and on to the three other secondary petioles, the pinnae of which also fold together from the base outwards. The secondary pulvini also take up the sleep position and shortly afterwards the primary articulation follows suit. But the process is not yet complete. After a short time the primary petiole of the leaf next above or next below suddenly droops and the stimulus soon affects the rest of the pulvini also. The stimulus may also be transmitted from the stem itself. If a deep cut be made in the stem, care being taken that it be not at the same time shaken, after a short interval movements appear in the neighbouring leaves. The stimulus may, when the conditions are favourable, be transmitted to a distance of half a metre, and the transmission is effected with a rapidity which, though indeed feeble when compared with the conduction of impulses in the nerves of animals, is very considerable as compared with other cases of like nature in plants. Various values have been recorded by different authors for the rapidity of transmission of the stimulus, and this is not to be wondered at, seeing that the plant is not always in the same physiological condition and that the stimulus may also vary ; still, allowing for the personal equation, there are many differences which are as yet inexplicable. At least it is certain that the stimulus is transmitted at the rate of several millimetres (2–15) per second ; by way of comparison we may remember that the speed of transmission in the nerves of the higher animals is about a thousand times as great, while the most rapid heliotropic response

observed as yet, viz. 0·3 mm. per second (*Brodiaea congesta*; ROTHERT, 1894, 137), is 10 to 50 times slower than the rate of transmission in *Mimosa*. Apparently the transmission of the stimulus in tendrils comes next to that of *Mimosa*, so far as rapidity is concerned.

One must not, however, compare the transmission of stimuli in the animal nerve with transmission in *Mimosa*, seeing that in the former conduction is effected by living protoplasm which is not the case in the latter. As a matter of fact, the stimulus in *Mimosa* may travel by way of tissues which have been killed by narcotics (PFEFFER, 1873 b) or by heating (HABERLANDT, 1890), and hence the conception of a transmission by living cells, and especially by intercellular protoplasmic strands, is excluded from consideration. DUTROCHET (1837) assumed that the stimulus travelled by way of the *vascular bundle*, and PFEFFER has endeavoured to confirm this view. The chief argument used was derived from an experiment of DUTROCHET's in which vigorous stimuli were propagated through a portion of a stem which had been deprived of its cortex, and in which the pathway available for the transmission of the stimulus was exclusively the vascular elements. PFEFFER endeavoured to show that the *movement of water* in the vascular tissue might serve as the medium of transference of the stimulus. Some of the water extruded from the pulvinus on stimulation might, he thought, enter the vascular bundle, and this sudden movement of water might be propagated along the vessels and so release a stimulus in other pulvini. It is immaterial how this movement of water arises provided only it be *rapid*, for movements of water connected with transpiration are well known to produce no effect at all. If an incision be made in the stem, the stimulus, as we have seen, is transmitted to neighbouring pulvini, but only when the vascular bundles are touched and when the extravasation of liquid from them demonstrates directly the movement of water taking place in them.

HABERLANDT's (1890) elaborate researches have completely upset this theory. This author showed that the sap referred to in PFEFFER's experiment did not by any means come out of the vessels, but from tubular cells in the phloem, corresponding to the tannin canals of other Leguminosae, but differing from them in the possession of numerous fine pores in their transverse walls. These pores allow of easy transference from place to place of the whole of the cell-contents, and every injury inflicted on such a cell permits an abundant extravasation of sap, just as in the case of sieve-tubes, and which may be recognized by its characteristic constituents. Movement also takes place when the stem is cut *only* when an alteration in pressure follows in these *tubular cells*, and such a change in pressure may be transmitted just as well in the dead as in the living tube. HABERLANDT has further shown that in DUTROCHET's decortication experiment the whole of the tissue, right into the wood, was not removed, but that the entire phloem along with the conducting hyphae was retained. These tubular cells HABERLANDT regards as the specific transmitters of stimuli in *Mimosa*. The one criticism, however, which militates against HABERLANDT's theory, is derived only from the result of the experiment where the cortex has been completely removed. This experiment showed that after the removal of the whole of the conducting tissue the stimulus could be transmitted by the xylem only. Although HABERLANDT has very ingeniously fitted this fact into the tail end of his theory by a subsidiary hypothesis, we are inclined to regard it somewhat in the light of the heel of Achilles! Further experiments specially directed to this point must show how transmission of the stimulus is effected when the *xylem* is interrupted but when the conductive tissue remains intact.

If this experiment should show that the xylem is indispensable for the transmission of the stimulus then we must fall back on the DUTROCHET-PFEFFER theory. One further point must, according to our idea, be cleared up in this theory, viz., how the liquid which exudes from the cells of the sensitive half of the articulation succeeds in entering the vessels. PFEFFER himself was

unable to observe any pressure worthy of the name in the intercellular spaces, when these were injected during stimulation. BONNIER (1892) even established a slight decrease in pressure after the stimulation by inserting a manometer into the pulvinus, arranged so as to show pressure of the air in the intercellular spaces. It is not easy to understand how, under such circumstances, the extruded liquid can enter the vessels *under pressure*. HABERLANDT'S views, on the other hand, are quite intelligible. On direct stimulation of an articulation, flaccidity ensues in the sensitive parenchyma and, owing to the deformation of the cells, a *pressure* will be induced on the conductive tissues which is propagated along them and which, wherever it reaches a new pulvinus, is capable of stimulating it just as if a blow had been inflicted on it from without. In that case we should have to consider this really as a genuine *instance of transmission of a stimulus and not of an excitation* as in other cases.

Other parts of the plant as well as the pulvini may be affected by a primary stimulus; for example, a pinna may be stimulated without touching its articulation (BERT, 1867), and yet it, too, is able to appreciate the stimulus. In this case, also, the cells conducting the stimulus must primarily become deformed, and an increase of pressure effected which is transmitted. Stimulus conducting cells are, as a matter of fact, also found in the pinnae, following the course of the larger vascular bundles. By employing the method of stimulation by wounding, BERT (1867, 17) showed that the *leaf parenchyma* was *insensitive* and that response took place only when the stimulus affected the *veins*. In the case of all stimuli, whether of the nature of an incision, scorching, or corrosion by acid, there was always a *decrease* in pressure in the conductive tissues, never an increase, but which was propagated just like increased pressure, and which led to *mechanical* stimulation in the pulvini. It would appear that increase and decrease of pressure might take place more readily in HABERLANDT'S thin-walled but turgescent tubular cells than in the rigid-walled vessels. [FITTING has (1903) carried out some new experiments on the transmission of stimuli in *Mimosa*, but this author has not been successful in solving the question in dispute.]

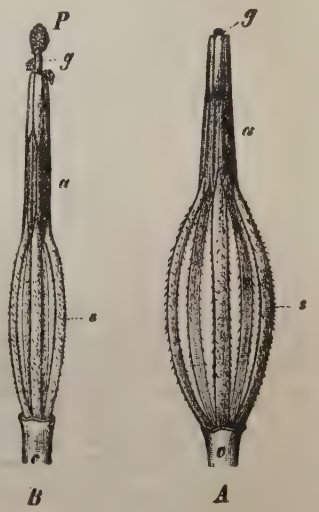


Fig. 160. Stamens of *Centaurea jacea*, after removal of corolla. *A*, before, *B*, after stimulation. *c*, base of the corolla; *s*, filaments; *a*, anthers; *g*, style; *P*, pollen. Enlarged. From the Bonn Textbook.

As far as regards sensitivity and the mechanics of movement in response to stimulus, a perfect comparison may be instituted between those phenomena as exhibited by *Mimosa* and those manifested by certain stamens, although the movements in the latter have an entirely different biological significance. Let us study the stamens of the Cynareae, more especially those of *Centaurea* (Fig. 160) which have been minutely investigated by PFEFFER. The five syngenesious anthers form a tube round the style; from these arise five free filaments, bent slightly outwards, the basal ends being inserted on the corolla lower down. When the filaments are touched (*A*) they contract and at the same time straighten themselves (*B*); in this way the anther tube is pulled downwards and the pollen is thus swept out by the hairs on the style; the movement is thus obviously an adaptation to pollination by insects. The filaments react *only* directly to the contact; there is *no transmission* of the stimulus. The experiment may also be performed on a single isolated filament, and it may be

noticed that the contraction, after stimulation, may amount to 10, 20, or even 30 per cent. of the original length. The filament has a very simple anatomical structure, consisting merely of a delicate vascular bundle surrounded by parenchyma, which latter is the only part that is sensitive. When stimulated, this tissue becomes less turgid and water enters the intercellular spaces, as in *Mimosa*, while the filament decreases markedly in volume. If water be previously injected into the intercellular spaces, the water exudes by the cut surface when the filament is stimulated, although in ordinary cases the intercellular spaces are sufficient to accommodate the liquid which has been extruded. In the Cynareae it may be definitely shown that a diminution in the expansion of the cell-wall is not concerned, since its elasticity is quite as great in the contracted filament as in the extended one, a fact which may be shown by previously subjecting it to the action of chloroform and so preventing stimulation from shock. Further, no change takes place in the condition of the membrane during the stimulation, for a weight capable of stretching a contracted filament out to its original length is also sufficient to prevent any contraction on stimulation (PFEFFER, 1873 a ; 1890, 326). On stimulation, therefore, there must be a decrease in the pressure of the cell-contents, apparently a decrease in osmotic pressure, which, according to PFEFFER, must amount to from 1 to 3 atmospheres. The plant therefore does not make use of the whole of the elastic play of the membrane, for the filament, after becoming contracted on stimulation, may be still further markedly shortened by plasmolysis, while, on the other hand, the filament when extended may still be stretched by mechanical means, within the limits of its elasticity. The cell-walls, when the tension is completely relaxed by plasmolysis, do not exhibit a permanent elongation, if they be extended 100 per cent. by mechanical means ; but in their extensibility, the filaments of the Cynareae stand quite alone in the vegetable kingdom (p. 420).

Sensitive filaments occur in other sub-orders of the Compositae besides the Cynareae (HANSGIRG, 1890), and curvature movements of filaments in response to a shock stimulus have been noted in Cactaceae, Cistaceae, Mesembryanthemaceae, Tiliaceae, Portulacaceae, and Berberidaceae as well. The filaments in these plants are sometimes sensitive on one side, sometimes on all sides, and the curvatures they exhibit are sometimes inwards, sometimes outwards; but in all of them the nature of the sensitivity and the method of movement appears to resemble the phenomena as exhibited by *Mimosa*. Transmission of stimulus has also been recognized in *Sparmannia* by HANSGIRG (1890). In most cases these movements have some obvious relation to the cross-pollination of the flowers.

In addition to single sensitive styles (*Arctotis* ; MINDEN, 1901) there are stigmas whose lobes approximate when stimulated by contact ; they occur especially in the Scrophulariaceae, Acanthaceae, Pedalineae, Bignoniaceae, and Capparidaceae; these movements have been as yet very little investigated from the physiological point of view. We are at liberty to assume, however, that the approximation in them also is the result of a decrease in osmotic pressure. In certain cases a transmission of stimulus takes place from one stigmatic lobe to another, but the conduction is obviously effected in a way altogether different from that seen in *Mimosa*. OLIVER (1897) supposes that the stimulus is transmitted by intercellular protoplasmic communication, at all events the transference of the stimulus is effected just as well after the vascular bundle has been cut through. After a simple contact, the stigma remains closed only for a short time, and after opening again it is ready to receive a new stimulus. BURCK has made the interesting observation that the stigma of *Mimulus luteus* remains closed if the contact be caused by pollen-grains ; *Torrenia fournieri* closes its stigma permanently if it comes in contact with pollen from the larger stamens, although it opens again if pollen from the smaller stamens or

from foreign plants be placed upon it. Closure of the stigma must thus be of service in excluding foreign pollen.

HABERLANDT (1901) has drawn attention to certain anatomical adaptations both in sensitive stigmas and stamens which are obviously correlated with reception of the stimulus, but we must refer our readers to his own descriptions of these.

Bibliography to Lecture XL.

BERT. 1867. Mém. Soc. des sc. phys. Bordeaux, 1866. Paris.
BERT. 1870. Ibid. February.
BONNIER. 1892. Revue gén. de bot. 4, 513.
BRÜCKE. 1848. Archiv f. Anatomie u. Physiologie. Ostwald's Klassiker, No. 95.
BURCK. 1901. Botan. Centrbl. 1902, 89, 645 Review.
CORRENS. 1892. Flora, 75, 87.
DUTROCHET. 1837. Mém. pour servir à l'hist. d. végét. et d. animaux. Paris.
[FITTING. 1903. Jahrb. f. wiss. Bot. 39, 424.]
HABERLANDT. 1890. Das reizleitende Gewebesystem d. Sinnpflanze. Leipzig.
HABERLANDT. 1901. Sinnesorgane im Pflanzenreich etc. Leipzig.
HANSGIRG. 1890. Botan. Centrbl. 43, 409.
HANSGIRG. 1893. Physiologische und phycophytologische Untersuchungen. Prag.
HILBURG. 1881. Unters. aus d. bot. Inst. Tübingen, 1, 23.
MINDEN. 1901. Flora, 88, 238.
OLIVER. 1887. Ber. d. bot. Gesell. 5, 162.
PFEFFER. 1873 a. Physiolog. Untersuchungen. Leipzig.
PFEFFER. 1873 b. Jahrb. f. wiss. Bot. 9, 308.
PFEFFER. 1890. Plasmahaut etc. Abh. K. Gesell. d. Wiss., 16, 185. Leipzig.
ROTHERT. 1894. Cohn's Beitr. z. Biologie, 7, 1.

LECTURE XLI

SUMMARY OF PARATONIC MOVEMENTS. AUTONOMOUS MOVEMENTS

IN our very first lecture we drew attention to the fact that it was not possible, off-hand, to recognize movement in all plants. Nevertheless, in so far as we have studied the changes in form and position exhibited by fixed plants, not to speak of the locomotory phenomena seen in non-fixed forms, we are bound to admit that the popular view that plants have *no power of movement* is entirely *erroneous*. Careful observation has made us acquainted with *abundant* instances of *movement*, although these are less noticeable than movements in the animal world, simply because they are *not so rapid*. Still, from the *scientific* standpoint, the *rapidity* of a movement is not the most important of its features ; its *nature*, its *causes*, the *means* by which it is accomplished, and its *significance* in the organic economy are the points to which our attention is most prominently directed. With regard to most of these points a remarkable parallel has been drawn in recent years between the movements in response to stimulus, described in Lectures XXXIII to XL, and the reflex movements of animals, and when, in Lecture XLIII, we come to consider locomotory directive movements the analogy will become even more remarkable.

In Lecture XXXIII we termed the various movements hitherto dealt with *paratonic, induced*, or *receptive* movements, and contrasted them with *autonomous* or *spontaneous* movements, and in that lecture also we briefly pointed out the grounds on which that distinction was based. Before studying autonomous movements more in detail we must endeavour to bring out the contrast more sharply than we have as yet done.

Movements in response to stimuli arise only as a result of the continuous

influence of the environment, and this influence is twofold; in the first place, it provides the *general* (*formal*) *conditions*, in whose absence no movement in response to stimulus—nor indeed any other vital phenomenon—can take place, and it also provides *specific stimuli*. Both these effects of the environment require further elucidation, and we may start with a consideration of specific stimuli.

We have already described these movements in response to stimuli as *released* movements (PFEFFER, 1893; Physiol. I, 9 and II, 80); it should be added that the factors which act as stimuli are merely the *inducing causes* of the movements which the organism carries out by its *own inherent energy*; the stimulus itself never provides the energy actually required for carrying out the movement. It follows that no definite relation exists between the energy of the stimulus and that manifested in the response. The response is always accompanied by an expenditure of energy, but the stimulus may be effected just as readily by a withdrawal as by an application of energy. An example of the former is seen in the stimulus induced by a reduction in temperature (compare p. 502). It may be said that the stimulus releases the reaction, it causes or induces it, and hence we speak of *induced movements*; we further ascribe to the plant the power of perceiving the stimulus or of being sensitive to it, hence the term 'receptive movements'. The stimuli we have previously dealt with have been *external* stimuli, such as light, heat, electricity, gravity, chemical and mechanical effects of certain substances, &c.; but, just as in the differentiation of the plant so in its movements there are *internal* stimuli concerned, some of which we have to study in this lecture.

Since the essential characteristic of a stimulus lies in the fact that it acts as a *releasing* agent, it follows that such stimuli are not confined to *organisms*; indeed we constantly make use of such releasing agents in *machines* of various kinds, and since the causal connexion is incomparably more apparent in them than in organisms, owing to the simplicity of the conditions, we can explain the principle of a releasing agent in a machine far more easily than we can in an organism (PFEFFER, 1893). Let us take the case of an electric bell. The apparatus consists of a bell, the electric battery, and the conducting wire. The bell is the mechanism which is stimulated by an electric current emanating from the battery. Ordinarily, however, the conductor which runs from the source of electricity to the bell is interrupted and a sound is produced only when the current is 'closed'. This 'closing' or joining of the wires is the point of interest at present, since it is the closing that 'releases' the bell's capacity for making a noise. In order to make and break the electric circuit a key is used, and the necessary metallic connexion between the terminals is made by means of a gentle pressure on a metal plug. It may be seen at once that the amount of pressure employed stands in no relation whatever to the loudness of the resulting clang. According to the way in which the key is constructed it may require the slightest touch of the finger or the whole of a man's strength to close the circuit, but, so long as the pressure is sufficiently great to effect closure, the bell responds in the same way, provided the electric current passing along the wire remains unaltered. When we remove the pressure from the button the key returns to its rest position, the current is interrupted, and the bell ceases to ring.

There would be no great difficulty in manufacturing other kinds of 'keys' by which the current could be closed by magnetic energy, electricity, heat, or light, instead of by mechanical means. It is quite unnecessary for us to spend time in describing how such an apparatus could be made, it will be sufficient for our purpose to note that a new type of key will be required for each type of releasing agent. No matter what the structure of the key, the bell reacts only to a single releasing agent; the mechanism responds to this influence, or, as we might say, is sensitive to it. It is also obvious that the application of pressure to any part of the system *other than* the key, i.e. the application of the releasing agent to any other place than the sensitive apparatus, must be quite ineffective.

This simple illustration will help us to understand many of the phenomena of stimulation. Pressure on the button no more provides the energy for moving the clapper of the bell than does contact in the case of a tendril, a shock in the case of the leaf of *Mimosa*, or gravity or light in geotropic and heliotropic movements respectively, carry out the work that is ultimately accomplished. It is quite true that when geotropism was first studied, attempts were made to prove that gravity was the actual source of the energy expended in the curving which ensued. What in the case of geotropism took many years and much hard work to demonstrate, was obvious on the face of it in the case of heliotropism. No one dreamt of suggesting that sunlight pulled the stem towards it and pushed the root away. Even the original explanation of heliotropic curvature given by DE CANDOLLE, and which we have described as a mechanical explanation, is not mechanical in the sense we now mean, for it always assumes a 'stimulating effect' of light.

Looking first at heliotropism by way of illustration, the heliotropic curvature or released response might be compared to the clanging of the bell in our mechanical illustration. The bell's capacity may also be regarded as mechanical; we can readily understand that such an apparatus, on account of its structure, is able to function in this way and in no other when an electric current affects it; on the other hand we do not know how it is that a plant *curves* when unilaterally illuminated, although we must assume that this phenomenon is the necessary result of the 'mechanical structure' of the plant, just as a clanging noise is the necessary consequence of the structure of an electric bell. That the active force in the case of the plant is turgor or growth and in the case of the bell electricity has nothing to no with the matter. The releasing force in the one case is pressure on the key, in the other case sunlight; with the sensitive apparatus in the machine we are fully acquainted but we know nothing of it in the organism. We can only say *where* the sensitive apparatus is in the plant, for we know that in many instances it is closely associated with the reacting region and in others that it is situated at some distance from it. Its structure apparently lies outside the limits of microscopic investigation and hence we can do nothing more than guess at its mode of operation. The same is true of all other stimulus phenomena; as to the structure and mode of action of the sensitive apparatus we are quite in the dark, although the conditions under which it carries out its functions have been more or less accurately determined.

Very many plants exhibit curvatures due to the influence of *other* stimuli, which differ from heliotropic curvatures in no respect or only in trivial details. Still, according to the nature of the stimulus, we have distinguished these movements as geotropic, chemotropic, thermotropic, and so on. These curvatures owe their origin to all appearance to the same mechanical structure as the heliotropic movements, but we must assume that the perceptive apparatus must have a different structure in each case, adapted to the reception of the particular stimulus in question, just as a different kind of key will be required in the electric bell apparatus according as pressure, electricity, light, &c., is the releasing agent employed.

Just as we meet with similar reactions in the plant accompanied by dissimilar perception, so the converse, also, holds good, for we find dissimilar response resulting from similar external influences. Thus the root responds positively, but the stem negatively, to gravity; again the same external factor may induce curvature in *one* organ and torsion in another, and the same differential illumination which induces *curvatures* may influence the *symmetry* of a plant, so as to induce the formation of new organs on one side rather than another, as, for instance, in the development of roots, roothairs, or sexual organs on the shaded side. There are two possible suggestions which we might offer by way of explanation of these phenomena. 1. We might assume that the plant possesses only one kind of perceptive organ by which it appreciates each individual

stimulus, but which is in connexion with different kinds of apparatus. This would correspond to the case where the electric wire, closed by the key, was connected up, in one case with a bell, in another case with a glow-lamp, and in a third with, let us say, a voltameter; the work done as a consequence of the same releasing stimulus would be quite different in each case. 2. It is also possible, however, that the difference in the reaction depends upon the varying structure of the perceptive organ. NOLL (1892) makes an assumption somewhat like this, and has accounted for the different forms of geotropic response in this way. There are many arguments against such a hypothesis, however, and we are inclined to think the first view is the more probable one.

Let us now inquire whether there is or is not only one kind of perceptive apparatus for each and every stimulating agent. The same stimulus can indeed induce a response in quite different ways. Light, for example, gives a stimulus to the plant when it affects the plant equally in every part, and the response given by the plant is indicated by alteration in its rate of growth. The response is totally different when the light falls with unequal intensity on opposite sides, say, of the shoot, for then the plant responds by exhibiting heliotropic curvatures. In contrast with these *regional* light stimuli we have the *periodic* variations in light intensity which lead to nyctitropic movements. The preliminary phenomena of stimulation in the case of heliotropism and nyctitropism are doubtless different from those which lead to etiolation. We have been compelled, for good reasons, to discard the 'etiolation theory' of heliotropism, and on similar grounds we are entitled to look with scepticism on any hypothesis which would seek to explain thermotropic or chemotropic curvatures by asserting that the organ in question exhibited growth in each longitudinal area with a rapidity proportional to the temperature to which it was exposed or to any definite concentration of the chemical medium employed. The facts are in striking opposition to such a hypothesis, for under certain circumstances the side that is more remote from the influence of the optimum temperature grows more rapidly than the others (compare p. 480). Although the phenomena precedent to stimulation in etiolated growth are doubtless different from those of heliotropism the first effect of light may be the same in both cases; we may assume, for instance, that in each cell a quantity of a certain material, proportional in amount to the intensity of the light, disappears with the discontinuance of illumination. This change, consequent on the action of light, may be compared with the closure of the key as a result of pressure, it indicates a preliminary chemical (or physical) action of the stimulant, and may represent what we term perception. If thereafter an acceleration or retardation of growth takes place in each sensitive cell, we must look upon that as the released movement. We have already especially noted that, in the case of etiolation, not all cells which perceive the stimulus proceed to react to it, otherwise all organs must elongate in darkness; experience teaches us that organs behave differently in this respect; *correlations* between the *individual units prevent a similar reaction of all.* All the same we may assume in this case that perception and reaction striven for are similar throughout; secondary influences, however, which we need not consider here, but which may be of a relatively simple character, may interfere with the reaction in certain regions.

The phenomena of heliotropism are different and more complicated. When perception, variable in its intensity, is brought about in different cells in consequence of unequal intensity of light, reaction does *not* follow it *directly.* On the other hand, the varying degree of sensation on opposite sides operates as a *new stimulus* and it is this that induces movement. Assuming that unequal sensitiveness in different regions acts as a new stimulus, we must grant to the plant the power of comparing the primary light effects in different situations. The term *comparison* suggests that we have here to deal with a psychical capacity in the plant. Although psychical capacity suggests *consciousness,* still we

must dismiss such an idea at once from our minds, for we are acquainted with plenty of examples of movement in our own bodies which take place without our being conscious of them, i.e. reflex actions, which, obviously, strongly resemble these responsive movements in plants. In the higher animals, however, an afferent conductive apparatus to a central organ and an efferent one to the motile organ are also required for the carrying out of the reflex action, in addition to the organ which perceives the stimulus (sense organ); the existence of such a central organ in plants has been suggested by CZAPEK (1898), but no reliable evidence in favour of the idea has been adduced, still less do we know as to its localization.

At present it is not easy to illustrate by means of a mechanism those movements at least which arise as a result of a comparison of differential primary stimuli. This much is clear, however, viz. that, in principle, such a machine must differ from that we have employed above, inasmuch as it must show two or more consecutive releasing movements before the chief or final response is observed. The electric current set up by closing the key must be made to release another current, or even another energy altogether, which in its turn does the work.

We may conclude, therefore, that the primary effects of light in etiolation and in heliotropism may be the same, and hence that it is sufficient to assume *one type* of perceiving organ for every stimulant, and this leads us to the conception that very frequently quite a *chain of releasing movements* may intervene between the first application of the stimulus and the final response.

Simple machines, like our electric bell arrangement, may still serve to render intelligible many phenomena of stimulation, such for instance as the relation between the intensity of the stimulus and the amount of the response. Looking at an ordinary electric key we see that a certain amount of releasing energy must be expended before perception follows. Every pressure which fails to make contact, fails also to reach the liminal value of the stimulus and induces no response, no matter how long it may be continued. Every pressure, on the other hand, which succeeds in making contact, induces a maximum reaction. The same thing occurs in *Mimosa*. In other stimulation phenomena, such as geotropism (pp. 439 and 457), we found that the response varied according as the releasing energy increased or decreased. It would not be difficult to construct a key of such a character that as pressure on it was increased, more and more electric elements became involved and added their currents to the total, so that the activity of the apparatus would thus bear a certain quantitative relation to the releasing force. The primary positive curvature of a heliotropic plant, followed by a negative curvature as the intensity of the light is increased, may be demonstrated by means of an electric model, where the key is pressed upon with ever-increasing force first on one side and then on the other. Such an apparatus also shows that the *regulating arrangements* seen in all stimulation phenomena, and which as a rule have a definite purpose to fulfil, are not characteristic of organic nature only; they apply to machines as well. We will not pursue these analogies further, however, for they are apt to mislead beginners into the belief that plant phenomena are simpler than they really are. We may, however, once more draw attention to the long *chain* of phenomena which is as a rule interpolated between the reception of the stimulus and the response (compare p. 440), and which in the machine is reduced to a minimum.

If we do not recognize, after all these remarks, at least one essential difference between the releasing stimulus in a machine and that in an organism, then the idea of a stimulus is to all intents and purposes superfluous. We retain it, in the first place, on historical grounds, but also because we are able, by its means, to recognize the *organism* at once as the scene of certain phenomena, while, at the same time, admitting that we are entirely in the dark with regard to the releasing process, as indeed to the entire chain of events right up to the final response (PFEFFER, 1893).

Just as in the case of every machine, so in the case of the organism, several conditions must be fulfilled if its various functions are to be carried out satisfactorily. In addition to *internal* factors there are also a number of *external* or formal conditions (PFEFFER, Physiol. II, 76) which must be taken into consideration, the significance of some of which is quite obvious, although the part played by others is as yet far from being understood. The need for water and certain constructive material is apparent on the face of it; oxygen also is required in order that respiration may be carried out, a physiological process without which, as a general rule, no movements can take place. In addition to these material conditions, heat and light must be taken into account, for a definite amount of heat, a definite degree of temperature, is one of the most fundamental formal conditions of plant life, while some, though by no means all reflex movements require light of appropriate intensity for their manifestation. Finally, injurious external influences must be absent, such as poisons and narcotics, which either merely delay the response or prevent it taking place altogether, or even may bring about the death of the organism, according to the degree of concentration in which they occur. Every insufficiency in any of the formal conditions tends to bring on a state of rigor, cold rigor, heat rigor, drought rigor, &c., and each of these rigors, if long continued, tends to become fatal.

None of these facts are new to us; we have summarized them once more merely that we may add to them a few general remarks. Let us inquire first as to the significance of the intensity of these factors. We have abundant data at our disposal on the subject, for in almost all vital processes we have established the fact that the influence of formal conditions is, in the most intimate way, dependent on their intensity, and that that dependence may be expressed graphically by means of a curve in which three cardinal points may be recognized, viz. a minimum, an optimum, and a maximum. It is often said that this dependence is limited to organisms, but that is by no means the case. It must be remembered that in the inorganic world also 'optima' may be recognized (compare ERRERA, 1896). Since water attains its maximum density at 4° C., we may express this dependence of density on temperature by means of a curve which exhibits one optimum point, but which certainly shows no minimum or maximum. There are also, however, processes, entirely apart from those occurring in organisms, which exhibit well marked minima, optima, and maxima in their relation to temperature. Thus the solubility of sodium sulphate has its minimum at 0°C., its maximum at 100°, and its optimum at 33° C. Betol (compare TAMMAN, 1898) reminds one even more of the organism, for it melts at 96° C. and, after cooling, remains liquid as long as it is maintained above +25° C. and under −5° C. Temperatures above the minimum (−5° C.) and below the maximum (+25° C.) do not act in the same way, for at 10° C. we reach an optimum, inasmuch as far more crystals make their appearance at that than at other temperatures.

Temperature influences the various processes in the plant in a variety of different ways; the graphic curve for assimilation is quite different from that for respiration or for growth, and we cannot be wrong in assuming that what is true of these processes is true of others. Naturally the behaviour of different organisms varies to a much greater extent still. Take the case, for example, of the dependence of responsive movements on oxygen only; we cannot wonder that typical anaerobes require no oxygen and may even be brought into a state of rigor by its *presence*, while the same pathological condition is induced in aerobes by its *absence*. It is a very remarkable fact, however, that among genuine aerobes, also, very great variations are met with in relation to the minimum percentage of oxygen required; CORRENS (1892) has shown that at least 6 per cent. of the normal amount of oxygen present in the atmosphere must be present before *Passiflora* can carry out haptotropic movement, while *Mimosa* leaves and the tentacles of *Drosera* will respond to contact stimulus when oxygen is entirely absent. The various phases in the reflex action also

exhibit dissimilar degrees of dependence on external formal conditions, and this fact enables us in certain cases to distinguish between perception, conduction, and reaction (compare p. 440) in cases where other criteria are not available. For example, geotropic response is inhibited by chloroform although its perception is not; in other cases the same anaesthetic may conversely destroy the power of perception of a stimulus and yet not interfere with the power of movement to any great extent (compare ROTHERT, 1903).

In conclusion, let us inquire how these formal conditions really operate. Do they operate in virtue of their own inherent energy or do they act as releasing agents only? In by far the majority of cases an exact answer cannot be given to this question; there can be little doubt, however, that certain essential substances, by bringing to the plant a store of energy or of constructive material, act as *energizing* bodies, while temperature and, generally speaking, the majority of the formal conditions are doubtless to be regarded as *releasing* factors only. How then are we to distinguish between formal conditions and specific stimuli? In many cases the distinction is undoubtedly possible, as, for instance, if the formal condition, e.g. temperature, is to be considered as a general stimulus for many or all of the vital processes, and the factor, regarded merely as a stimulus, may be proved to induce a single change or movement. In other cases no such distinction can be drawn, for when several external conditions are operative at the same time it is exceedingly difficult to say which of them are to be considered as formal conditions and which as specific stimuli. An example will make this clear. A lateral root in darkness assumes a different geotropic rest position from what it does in light. If the roots be first of all grown on a klinostat and then allowed to carry out a geotropic curvature, we must look on *gravity* as the stimulus and say that the result of this stimulus is different according as it operates on an illuminated or a darkened root. If we allow the root, however, to attain its geotropic rest position in the dark and then illuminate it, we must conclude that the *light* is the *stimulus* which brings about the ensuing curvature. Similar cases are of very frequent occurrence.

If we regard all the formal conditions as operating with optimum intensity, and if care be taken that they remain constant for a long time, and that other external influences are entirely excluded, the movements in response to stimuli hitherto studied cannot take place; but it would be quite incorrect to assume that the plant under these conditions was incapable of movement. In the first place, it is obvious that the conditions which we have assumed are operating are just those which are favourable to growth, and all growth is necessarily accompanied by movement. Although many plant organs when exposed to uniform external conditions grow more or less in a straight line, there are other organs also which exhibit *growth curvatures* without any *specific* external stimuli being applied, which are very similar in character to curvatures in response to stimuli already studied. Variation curvatures, however, do not, in general, cease when specific stimuli leading to curvature are absent. Growth and variation movements, which cannot be referred to definite external stimuli, but which are dependent on formal conditions just as are the reflexes, are spoken of as autonomous or spontaneous movements. Each movement must naturally, all the same, have its own specific *cause* and the term 'spontaneous' must not be taken as synonymous with 'causeless'. If external factors be excluded from consideration as the agents to which these movements are due, *internal* factors must be assumed in their place. When we further investigate what these internal causes in turn really are, it would appear in the highest degree probable that we are dealing here also not with energizing but with *releasing* agents; in other words, spontaneous movements are to be considered also as *stimulus-movements*, although the stimuli inducing them are not *external* but *internal* and *unknown*. Although we have contrasted spontaneous movements

with movements induced by stimuli previously studied, this comparison does not by any means lead us to the root of the matter. We cannot prove it, but we think it is extremely likely, that autonomous movements are also induced, but by *internal* rather than external stimuli. It will now be our task to attempt to gain some acquaintance with these autonomous movements. As already noted, we may distinguish them into variation movements and nutation movements, according to the media by which they are carried out.

When speaking of nyctitropic movements of articulations we had occasion to note that these periodic oscillations continued for a long time in darkness when the temperature was kept constant, with almost the usual daily rhythm ; in that case we were dealing with *after-effects*, which must not be confounded with autonomous movements. These oscillations are very prominent in *Mimosa* and *Acacia*, but they are not manifested by all leaves provided with pulvini. When we study a plant of clover kept in darkness, we may observe very marked to and fro oscillations in the leaflets which, however, show no relations to daily periods (PFEFFER, 1875). This is a case of *genuine* autonomous movement which is indeed itself autonomously *periodic*. These movements occur in light also, though frequently masked, owing to the greater effect of the paratonic (nyctitropic) movements. In *Averrhoa bilimbi* (a member of the Oxalidaceae) these movements may be seen very clearly. When temperature and illumination are kept constant the pinnate leaves of this plant continually perform backward and forward oscillations (DARWIN, 1881), suddenly drooping and then slowly rising again. [Very remarkable autonomous movements are also exhibited by *Oxalis hedysaroides* (MOLISCH, 1904).]

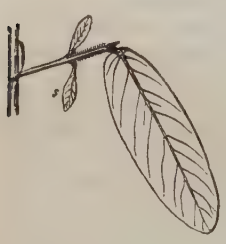


Fig. 161. Leaf of *Desmodium gyrans*. Reduced. After PFEFFER from DETMER'S Practical Physiology.

PFEFFER'S (1875) researches have shown that while the autonomous movements are in progress, just as in the case of after-affects, the resistance to flexion of the pulvinus remains *unaltered*. We may, therefore, assume that the expansive force of the cells on the concave side of the articulation decreases proportionally as it increases on the convex side.

The leaves of *Averrhoa* and of the majority of nyctitropic plants perform simple autonomous pendulum oscillations, but in the well-known *Desmodium gyrans* (DARWIN, 1881, p. 304) the movements are more complicated still. The leaves of this plant (Fig. 161) are tripartite. The terminal leaflet is large and performs well marked nyctitropic as well as less noticeable autonomous movements, while the two smaller lateral leaflets, on the contrary, show no nyctitropic movements, but do show autonomous oscillations, which at a certain temperature (minimum 22° C.; optimum, 40° C.) are so rapid that they may be readily followed with the naked eye. The whole backward and forward movement is complete in about 1½ minutes. The alteration in the expansion of the pulvinus does not take place alternately, first on one side and then on the other, but it proceeds in a circular manner, one longitudinal area after the other being affected. The result of that is that the tip of each leaflet describes approximately an ellipse whose long axis is parallel with the main petiole. The movement is, however, not uniform but is jerky in character, and on the whole more rapid downward than upwards. The jerks are especially prominent if the efforts to move on the part of the leaf are prevented for a long time by external resistance, so leading to tissue tensions. According to STAHL (1897), such tensions, arising by inhibiting movement in the terminal leaflet, as they become equalized, lead to vibrations and hence to increased transpiration in the terminal leaflet. Whether other autonomous variation movements also have a biological significance may be left undecided.

In the flowers of certain Orchidaceae and Stylidiaceae we also meet with many

remarkable autonomous movements which are perhaps of the nature of variation movements. In *Stylidium adnatum* (GAD, 1880) it is the gynostemium which oscillates and which occasionally presses itself so vigorously against one definite perianth leaf that tensions arise which in the long run may lead to a sudden release of the column from the leaf; the sudden backward curvature thereby arising resembles in its character a reflex action. [Compare HOSSENS, 1903.] Among Orchidaceae oscillating movements occur in *Megaclinium falcatum* (MORREN, 1842) which are in this case carried out by a narrow basal part of the labellum, but of whose mode of operation we know nothing. Possibly it may be a growth movement, at least autonomous periodic movements are much more frequently due to growth than to changes in turgidity.

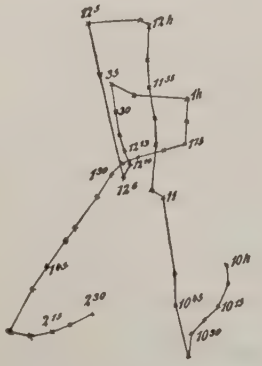


Fig. 162. Projection curve of *Phycomyces nitens*, after FRITZSCHE (1899). × 200.

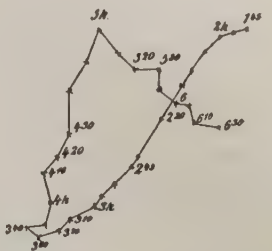


Fig. 163. Projection curve of *Zea mais*, after FRITZSCHE (1899). × 10.

The whole cycle of growth under constant external conditions, i. e. the grand period so called (Lecture XXIII), may with perfect correctness be regarded as an autonomous movement. During that period the apex of the root or the stem does not follow a perfectly straight course (circumnutation; DARWIN, 1881). Where such apices do appear to grow straight, looked at casually, the microscope discloses inequalities in growth in certain longitudinal areas which

Fig. 164. *Yucca filamentosa*, showing two axes of inflorescence. *I*, May 27, 1900, noon. *II*, May 28, 9.30 a.m. *III*, May 28, 2.30 p.m. From a photograph. Reduced.

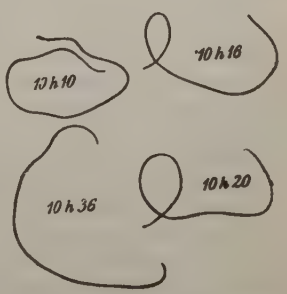


Fig. 165. *Spirogyra princeps*. Positions taken up by a filament at short intervals. After HOFMEISTER, 1874.

are sometimes regular, at other times follow no obvious rule (FRITZSCHE, 1899). In Fig. 162 the movements of the conidiophore of *Phycomyces* are recorded, as observed from above by means of the microscope. If the conidiophore grows rectilineally its apex must always occupy the same position in the field of the microscope, but in reality readings taken even after 7½ minutes show that it has moved away considerably from that position. Similar records may be obtained

by observations in seedlings (i.e. *Zea mais*; Fig. 163). The nutations performed by many of the higher plants are still more complicated and more irregular if several zones of growth come into operation at the same time. The movements of the inflorescence of *Yucca*, for example, are very remarkable; indeed during the process of unfolding the inflorescence looks as if it were pathological, but by and by it assumes its normal straight character. Fig. 164 shows these changes in position. Curvatures also arise in the cellular filaments of the Zygnemaceae, due to the irregularities of growth which are always taking place and which cause the filaments to assume different positions. Fig. 165 shows a filament of *Spirogyra* drawn at short intervals (compare E. WINKLER, 1902).

It is impossible for us to enumerate all the cases of autonomous nutation referred to in the literature on the subject; we must confine ourselves in what follows to examples of especially uniform nutations.

The best known are the *revolving* nutations (NOLL, 1885; WORTMANN, 1887), where the oblique or horizontal apices of plants move forward in circles or ellipses. This type of nutation corresponds exactly to that shown by *Desmodium*, but it arises from the fact that one flank *grows* more vigorously than the other and that this increment of growth affects new longitudinal areas in regular succession. Circumnutation may be compared also with the outwardly similar movements of the apices of twining plants, and here, as there, we find a twisting of the apex of the shoot on its long axis in order to avoid torsions. The difference between the two sets of phenomena lies in this, however, that whereas in the case of twining, gravity plays an active part, we have here to do with purely autonomous movements. Such movements occur very prominently in tendrils and are obviously of the utmost importance as aiding them in finding a support. Circumnutatory movements occur also in seedlings, stola, &c., and, in these cases, when the curve is a much elongated ellipse, they approach the *pendulum* movements in character, as may be especially well seen in *Allium scorodoprasum*. Careful observation certainly shows that in the case of pendulum movements the oscillations are by no means always in the same plane, any more than, in circumnutation, are true circles or ellipses described by the growing points. Hence it would be difficult to separate these two types of nutation from each other.

The same is true of a further distinction which has been drawn between *periodic* and *transitory* nutations. Typical transitory or solitary nutations occur in many organs which are curved at an early stage in their growth and afterwards become straight. The cotyledons, the hypocotyl, and the root of the embryo frequently exhibit special curvatures characteristic of the type, and which appear to be entirely autonomous. With these may be associated the curvature manifested by embryonic organs in the bud. Stamens, floral and foliage leaves are very frequently curved inwards owing to excessive growth on the under side (hyponasty), and become straightened later on, owing to increased growth on the upper side (epinasty). The bud form is thus due to hyponasty of the lateral appendages. Not infrequently it happens that the epinastic outcurvature is not completed in one movement, but that a more vigorous depression alternates with a feebler erection, thus constituting a periodic nutation. Transitions between transitory and periodic nutations occur in those cases where epinastic growth, so to speak, shoots beyond the mark, as, for example, when the leaflets of *Aesculus*, converging upwards in the bud position, bend downwards on the opening of the bud and then, during unfolding, owing to renewed hyponastic growth, spread out approximately horizontally. Epinasty and hyponasty cooperate with diageotropism and diaheliotropism in bringing about the definite rest position of dorsiventral organs. External factors often aid in bringing about this result, or they may operate antagonistically to it. It is impossible for us to discuss the action of epi- and hypo-nasty in greater detail; the matter becomes especially complicated, inasmuch as in addition to autonomous movements induced nastic movements also come into play (e.g. photonasty).

Peculiar nutations are met with in the leaves of ferns and of other plants whose leaves retain the power of apical growth for a long time (e. g. *Drosophyllum*; compare GOEBEL, Organographie, p. 508, Fig. 336). Such organs have their apical regions coiled in a circinate manner. In ferns this circinate condition is *hyponastic* and, as the leaf straightens itself, a less vigorous *epinastic* curvature sets in, ultimately bringing about the expanded condition. Similarly, in the case of the nutations of many seedlings, where more vigorous elongation takes place on one side of the plumule, usually spoken of as the posterior side, a second curvature occurs on the incurved apical region at the zone of maximum growth which acts antagonistically to the original curvature. WIESNER (1878) speaks of an 'undulating' nutation in this case, and of a 'simple' nutation when the region of the plumule behind the hooked end is straightened at once (*Linum*).

In addition to autonomous movements in one *plane* we have also autonomous movements in *space*, such as torsions and twinings. Examples of this type of movement are met with in the peduncles of *Vallisneria* and of many species of *Cyclamen* after fruiting, leaves of the garden variety of *Juncus* known as *Juncus spiralis*, leaves of *Typha* and many other narrow-leaved Monocotyledons, the labellum of *Himantoglossum*, the internodes of *Chara*, and finally the senile coilings of tendrils previously mentioned. We must limit ourselves to the mere enumeration of such instances for they have not as yet been studied in detail. It is not improbable that it may be found necessary to remove many of these examples from the category of autonomous to that of induced movements, as has already been done in the case of the nodding flower-bud of *Papaver* and the apex of the shoot of *Ampelopsis*; such cases of 'nodding' would certainly have been regarded as autonomous from their likeness to the nutation of plumules had it not been that VÖCHTING (1882) and SCHOLTZ (1892) have shown that they are geotropic in their nature.

Glancing back over what has been said, we recognize in autonomous movements phenomena which, as yet, are very imperfectly understood both from the physiological and from the biological point of view—hence the brevity of our treatment of them.

Bibliography to Lecture XLI.

CORRENS. 1892. Flora, 75, 87.
CZAPEK. 1898. Jahrb. f. wiss. Bot. 32, 175.
DARWIN. 1881. Das Bewegungsvermögen d. Pflanzen (CARUS). Stuttgart.
ERRERA. 1896. L'optimum (Revue Univ. Bruxelles, 1.)
ERRERA. 1900. Génération spontan. (Revue Univ. Bruxelles, 5.)
FRITZSCHE. 1899. Beeinfl. d. Circumnutation d. versch. Einfl. Diss. Leipzig.
GAD. 1880. Bot. Ztg. 38, 216.
HOFMEISTER. 1874. Würtemb. naturw. Jahreshefte.
[HOSSENS. 1903. Beeinfl. d. auton. Variationsbewegungen d. e. äuss. Faktoren. Diss. Leipzig.]
[MOLISCH. 1904. Ber. d. bot. Gesell. 22, 372.]
MORREN. 1842. Mém. Acad. Bruxelles, 15.
NOLL. 1885. Bot. Ztg. 43, 664.
NOLL. 1892. Heterogene Induction. Leipzig.
PFEFFER. 1875. Periodische Bewegungen. Leipzig.
PFEFFER. 1893. Reizbarkeit d. Pfl. (Gesell. d. Naturf. u. Aerzte. Verhandlungen).
ROTHERT. 1903. Jahrb. f. wiss. Bot. 39, 1.
SCHOLTZ. 1892. Cohn's Beitr. z. Biol. 5, 373.
STAHL. 1897. Bot. Ztg. 55, 71.
TAMMAN. 1898. Quoted by Errera, 1900.
VÖCHTING. 1882. Bew. d. Blüten u. Früchte. Bonn.
WIESNER. 1878. Sitzunsgber. Wiener Akad. 77, 1. Abt.
WINKLER, E. 1902. Krümmungsbewegungen von *Spirogyra*. Diss. Leipzig.
WORTMANN. 1887. Bot. Ztg. 45, 49.

LECTURE XLII

AUTONOMOUS LOCOMOTORY MOVEMENTS

HAVING discussed the movements exhibited exclusively by fixed plants, we must now turn to movements in those plants or plant parts which are capable of locomotion from place to place. At the first glance it would appear as if we had to deal in such cases with an entirely different category of phenomena from those we have hitherto been considering. More careful study leads us, however, to the conclusion that it is only the nature of the reactions, i.e. the change of place and the apparatus by which these reactions are carried out, that alone are novel. The general and special conditions under which locomotion is effected are on the contrary essentially similar to those we have met with already in the phenomena of growth and movement in fixed plants. The external stimuli which influence the nature of the response are precisely the same immaterial and material agents referred to in previous lectures on movements in the higher plants. Indeed many authors have advocated the treatment of locomotory movements simultaneously with those of curvature. While keeping these two series of phenomena apart in our present discussion on the subject, attention must be drawn to the numerous analogies which exist between them, further instances of which we shall meet with frequently enough later on.

We will first of all study *autonomous locomotory* movements which stand in close relation to the autonomous curvatures considered in the last lecture; *induced* locomotory phenomena will be treated of in the next lecture.

Autonomous locomotory movements are exhibited by the protoplasts of almost all plants, but they are naturally limited in extent by the rigid cell-walls. In many lower organisms, on the other hand, these movements are not so *circumscribed*, for such organisms have the power of creeping over the substratum or of swimming through the watery medium in which they live. We will take the latter case first.

Natatory movements occur in many Flagellata, very lowly organisms which may with equal accuracy be classified either among plants or among animals. These movements may continue during the *entire life-history* of the organism. In Algae, Fungi, and Bacteria, certain cells, at least *temporarily*, possess the power of locomotion; such cells are known as swarmspores or zoospores, and their function is to carry out asexual propagation and thus conduce, more especially, to the wide distribution of the species concerned. Further, the sexual cells are frequently adapted to a motile existence, both male and female cells among the lower forms having that power, while among more highly developed types motility is confined to the male celi. Motile sperms occur not only among mosses and ferns, but the corresponding cells even in Gymnospermae may exhibit the same characteristic (of motility) more or less distinctly. All such motile cells are provided with filamentous appendages, flagella, or cilia, whether they be surrounded by cell-walls (Bacteria, Flagellata) or not (swarmspores, sperms). These cilia effect the movement of the cell-body by rapid bendings, beating the water and driving the cell forwards, as a boat is propelled by its oars. They are developed from the ectoplasm and are themselves protoplasmic in character. In order to carry out their function they must be surrounded by water, into which they project through pores in the cell-membrane. As a general rule alterations in form do not come under consideration in natatory movements.

Let us take as our first example of an organism propelled by cilia the swarmspores of Algae (NÄGELI, 1860). The swarmspores are naked, and formed, several at a time, in a mother-cell, and each exhibits all the essential constituents

of the typical cell, i.e. protoplasm, nucleus as well as chloroplasts. They are almost always elongated, ovoid or pear-shaped, always markedly polar, but by no means always exhibiting radial structure in relation to their long axis. One pole, the anterior one in any movement, is generally free from chlorophyll and provided apically, or not infrequently laterally, with two, four, or more cilia. The posterior end is generally more rounded and contains chloroplasts. The movements are by no means simple, consisting as they do not only in a forward movement in the direction of the long axis of the cell but also a *torsion* on it. This is at least true in the case of certain cells ; in other instances the movement is more complicated still. The forward movement may, instead of being in a straight line, be in the path of a long drawn out spiral, the body of the cell rotating on its axis at the same time, the axis remaining parallel to that of the spiral. Finally a third type of movement is met with, when the anterior end of the swarmspore advances in a spiral manner while the posterior end maintains a straight course. If there be no mechanical or stimulating interference to its movements the swarmspore continues to follow the direction taken at the commencement of its movement, which on the whole is approximately a straight line, but in other cases the spores swim in curves or move about quite irregularly.

When the swarmspore meets with a mechanical obstacle it is able without moving from the spot to institute a twisting movement, frequently recoiling or moving backwards, twisting on its axis in the opposite direction. The butt end is anterior in this movement but very soon the original forward movement is resumed. Apart from the backward movement, the torsional movement changes only in certain free swimming cells ; in the majority of cases the direction of torsion is constant and characteristic of the species.

All these phenomena may be observed in swarmspores only if these movements be retarded, and this is best effected by replacing the water-culture by a weak solution of gum. The absolute rapidity of movement of the swarmspores, which is markedly dependent on external conditions, is by no means great. It appears to be considerable only when looked at under the microscope, but then it must be remembered that the *distances are also magnified.* According to HOFMEISTER (1867) the movements are most rapid in the swarmspores of *Fuligo varians*, viz. about 1 mm. per second ; the swarmspores of *Ulva* attain a speed of 0·15 mm. per second (STRASBURGER, 1878), but the antherozoids of the fern move much more slowly—0·015 to 0·030 mm. per second—according to PFEFFER (1884).

The fern antherozoids, which we shall study more in detail in the next lecture, differ from the zoospores of Algae in their form only but not in their movements. They consist of a spirally twisted body with 2–4 coils (Fig. 107, p. 359), tapering gradually from base to apex, the cilia being inserted on the thinner anterior spirals. No alteration in the form of the cell takes place here either during the movement.

That the cilia are the *agents* concerned in the movement may be easily proved. If a swarmspore be cut in two, only the part bearing the cilia remains capable of movement. If, by mechanical means, the cilia be removed, all movements cease and the body of the swarming cell sinks to the bottom. *How* the cilia bring about the forward movement, and, at the same time, the torsion, has not been investigated in detail, but we can scarcely be mistaken if we assume that the lashing of the cilia are carried out in the same way as VERWORN (1901) has demonstrated in the case of the ciliate Infusoria (Fig. 166). The apex of the cilium is seen at first to lie parallel with the forward path of the animal and, in the left-hand diagram (Fig. 166), the successive positions taken by the cilium at short intervals are figured ; the withdrawal (right-hand figure) is effected by *other* curvatures which are much slower, otherwise a forward movement would be impossible. The rotation of the body is due to the fact that the curving of the

cilia does not take place in one plane. When several cilia co-operate in effecting the forward movement (e. g. *Oedogonium* and *Vaucheria*) they must obviously beat at the same rate, otherwise the movements would be quite irregular.

In addition to *natatory* movements the lower organisms also exhibit *creeping* modes of locomotion, necessitating at all events a partial adherence of the body to the substratum. In some cases these movements are due to the exudation of slime from the cells (Desmidiaceae, STAHL, 1880; ADERHOLD, 1888: *Oscillaria*, CORRENS, 1897: and possibly also Diatomaceae, O. MÜLLER, 1897: compare also LAUTERBORN, 1896, and SCHÜTT, 1899). Putting on one side such movements as these, which have not as yet been examined in detail from the physiological standpoint, we have left for consideration the locomotory movements of naked protoplasmic masses, which creep over the substratum by *alterations in their form*. Such phenomena are spoken of as amoeboid movements, since they were first exactly studied in Amoebae; in the vegetable kingdom they are exhibited almost solely by the slime Fungi (Myxomycetes). The swarmspore, after escaping from the spore-wall, moves partly in an amoeboid manner partly by means of a flagellum; later on many of these bodies fuse together into the so-called plasmodium, which continues to exhibit amoeboid movement until the recurrence of the reproductive stage. Owing to its large size, the plasmodium is eminently adapted for the study of amoeboid movement, for all the phenomena can often be observed with the naked eye without calling in the services of optical instruments. Such plasmodia, especially those of the Physareae, (Fig. 167), occur on decaying leaves and old tan in the form of much-branched, reticulate filaments of very varied thickness, the more delicate anastomoses being visible only with the aid of a microscope. DE BARY (1864, p. 37) gives the following account of the general appearance and movements of the plasmodium:—'In one region, the anterior or advancing side of the plasmodium, the chief branches are especially richly subdivided and the terminal twigs are swollen at their ends and spread out in a fanlike manner over the substratum, and united by very numerous anastomoses. The individual branches and anastomosing threads of the anterior peripheral network are either thick, hemispherical or circular in section, with club-shaped, swollen and often ragged ends, or they may be flattened out so that the advancing border is thin and perforated, with indented margin and somewhat lobed, the whole being permeated by the stouter branches, like the swollen veins in a mesentery. The plasmodium is soft and slimy in its texture and may be readily smeared out with the finger, and yet it is firm enough to be sectionized with a sharp knife, so as to show the cut edge. Most commonly it adheres firmly to the substratum, but if placed under water large portions separate off, without suffering injury, in soft, elastic but by no means liquid masses.'

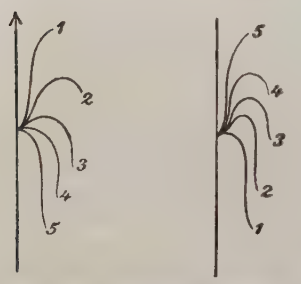


Fig. 166. Movements of single cilium of a ciliate Infusorian. After VERWORN (General Physiology, Jena, 1901).

'It may be readily seen with the naked eye that the plasmodium is continuously altering its shape, new branches being pushed out and others gradually retracted, so that the whole body slowly creeps forward.' This may be seen even better, however, under the microscope. 'The chief branches are constantly increasing and decreasing in thickness, every here and there broad processes appear on their upper surfaces which slowly or rapidly develop into new branches, while branches already in existence become gradually reduced until they are reabsorbed into the main body. Two branches may be seen growing towards each other, until their ends touch, and the next moment the anastomosis is complete. A lacelike network may thus arise at any point, composed of fine, threadlike uniting strands. If the anastomosis be broken, the two branches are slowly reabsorbed

into the main axes. These movements are much more prominently exhibited by the microscopic branches than by those visible to the naked eye ; such filaments are being perpetually pushed out and pulled in, just like fine tentacles, and the shape of the plasmodium is thus constantly altering ; branches shoot out and are withdrawn again, forming and breaking anastomoses, often swelling up to a great size and gradually taking on the characters of the stouter main branches. These alternative movements may be observed in all parts of the plasmodium, but they are readily seen to be more vigorous on the advancing side than behind, and that, anteriorly, the characteristic feature is the protrusion of new branches, while, posteriorly, the reabsorption of older strands is predominant ; hence arises the forward creeping movement of the plasmodium.' The direction of the motion is, however, not infrequently changed.

Fig. 167. Plasmodium of *Fuligo varians*, creeping over a piece of filter paper. From a photograph. Slightly reduced.

In addition to this change in outward form associated with regional changes of the whole plasmodium, other active movements in the interior of the plasmodium may also be observed. The plasmodium consists of a colourless, hyaline ground substance, the protoplasm proper, through which are scattered numerous granules, some of them composed of carbonate of lime, others of pigments. The streaming movements of the protoplasm are easily followed by the passive migration of these granules. Thus we may observe, first of all, in the centre of each branch an active streaming taking place, while the peripheral region, on the contrary, is at rest, not only the hyaline edge but layers still further inwards in which granules occur. The movement takes place as though in a tube, for a time in one direction and later in the opposite direction. In the marginal prominences for the most part numerous streaming movements may be noticed, and not infrequently contiguous portions move in opposite directions. Very often movements arise in regions previously at rest, so that one may assume that canals in which the streaming occurs did *not* exist there. This is shown even more clearly by the streaming motion spreading laterally, the previously firm hyaline wall becoming fluid and mobile. Rapid movements of the granules always take place, especially in the active anterior regions of the branches, as though this streaming were the cause of the forward movement of the tip of the branch. The smaller

projections, however, consist frequently of hyaline protoplasm only, destitute of granules, so that the interdependence of internal granular streamings and external alterations in form is somewhat doubtful.

The simplest form of amoeboid movement is met with in the Amoebae. *Pelomyxa*, for instance, consists of a flattened, elongated mass of protoplasm, which creeps over the substratum without any great change in form. Centrally we may observe a single axial stream of granules spreading outwards towards the advancing regions of the body of the organism, and the granules in the rear end appear to converge into this axial current, while a zone surrounding the body where the granules are *at rest* appears to separate the region of confluence behind from the region of effluence in front. *Amoeba* behaves exactly in the same way (Fig. 168); a forward movement appears not only at *V*, but also in the laterally advancing branches at *L* and *R*, so that the granules are seen streaming in three directions. Between *V* and *R* a fourth, but subsidiary, current may even be noted, so that there are five zones (indicated by crosses) which are at rest.

The detailed description of amoeboid movement given above renders the phenomena of movement seen in the protoplasts of the higher plants, which are enclosed in cell-walls, easily comprehensible (HOFMEISTER, 1867). We might, in fact, compare such protoplasts with Myxomycetes enclosed by a membrane. A *stationary* layer of protoplasm of greater or less thickness always lies immediately in contact with the wall; next comes a layer of motile protoplasm lying between the vacuole and the peripheral layer, and from this motile layer arise the anastomosing strands and filaments, similar to those seen in a plasmodium, which permeate the cell-sap as with a network and which exhibit continual alterations in form and position, although these, owing to the confined character of the cell cavity, are necessarily limited in extent. Just as in the case of the plasmodium, so here also we observe a streaming of granules both in the individual strands and in the peripheral primordial utricle. The direction of these currents varies from time to time and, even in contiguous regions of a strand, may be not infrequently in opposite directions; in other cases the granules may accumulate all on one side.

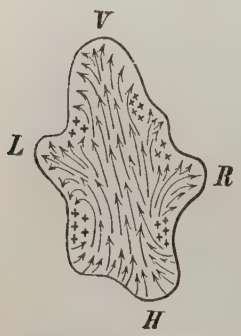


Fig. 168. Movements in *Amoeba*. The arrows indicate the direction and rapidity of the streaming; the crosses mark places where no movements are taking place. From the Bonn Textbook.

In addition to these more irregular protoplasmic movements, another type of movement has been distinguished from those just described (*circulation*) under the name of *rotation*. In this type of movement the peripheral protoplasm (save an external layer of varied thickness) moves in a constant direction, following, in elongated cells, the long axis of the cell, and often showing obvious torsion if the cell be exceptionally long. The movement is most rapid nearest the vacuole, which becomes moved about passively, showing that the cell-wall and the vacuole are to the protoplasm of the cell what the substratum is to the plasmodium. The vacuole acts as a *pivot* for the movement, and hence the current set up in it is in the reverse direction to those in the protoplasm.

It is frequently the case that, in addition to setting the minute non-living particles included in the protoplasm in motion, rotation and circulation also bring about, passively, alterations in position of the organs of the cell, i.e. the nucleus and the chloroplasts, and these changes in situation are often of great importance in the plant economy.

Owing to the widespread occurrence of the movements described and to their obvious importance, attempts have for long been made to discover the factors concerned in them, and not only to correlate amoeboid with the obviously related rotatory and circulatory movements, but to include under the same

category ciliary and muscular movements as well. From the well-known characters of muscular motion we might feel inclined to refer all these protoplasmic movements to contractility of the outer layer. When such an assumption was found to be indefensible, HOFMEISTER (1867) attempted to show that protoplasmic movement was due to a change in the attraction for water of the smallest protoplasmic particles, while ENGELMANN (1879) held that it was due to alterations in their form. All these attempts to explain the phenomena attribute to the protoplasm an unexplained character, which is taken for granted, although it must apply to living protoplasm only and not at all to lifeless bodies; moreover, they remove the problem into the realms of the invisible. More recent explanations (compare JENSEN, 1902), such as those of BERTHOLD (1868), BÜTSCHLI (1892), and QUINCKE (1888), must be dealt with more carefully, because they attempt to refer protoplasmic movement to purely physical causes.

In general these theories assume that protoplasm is a *liquid* and that its normal shape is a sphere. Variations from the spherical form and the movements themselves are thus accompanied by alterations in the *surface tension* [compare EWART, 1903]. As a matter of fact, the protoplasm, after receiving a wound or other injury, may frequently be observed to round itself off into a sphere, and hence it cannot be doubted that certain parts of it at least are liquid in character. Surface tension is undoubtedly a very important principle, but we must not expect to solve by its aid, once for all, every problem connected with protoplasmic movement. Moreover the authors referred to are not in accord as to the details of the explanation.

In order that we may obtain a superficial acquaintance, at least, with such physical theories of protoplasmic movement, we will look somewhat more closely at amoeboid movement only, and leave on one side ciliary motion and the streaming that takes place within the cell, since these present greater difficulties. We need not discuss QUINCKE'S (1888) views, since they assume certain conditions that are certainly not realized in the organism. We will therefore limit ourselves to a consideration of BERTHOLD'S (1886) and BÜTSCHLI'S (1892) theories as to the movements exhibited by such a form as *Pelomyxa*. BERTHOLD compares this amoeba with a drop of liquid spreading itself over, or round, a solid body or a drop of another liquid with which it cannot mix. Looking more especially at the former case, and considering a drop of liquid lying on a plate of glass, the space which it covers will depend in the first instance on the surface tensions between the glass and the liquid, the glass and the air, and the liquid and the air, and this will vary especially according to the chemical composition of the liquid and with the temperature. A homogeneous liquid spreads over the substratum equally in the form of a lens; if the glass be not quite clean, or if the drop be heterogeneous, or if it be of a different temperature in different places, the form of the drop is irregular and more extended in *one* direction than another. In *Amoeba*, owing to chemical differences between anterior and posterior ends, polarity is induced; the anterior end alone extends in a thin layer, adhering to the substratum, while the posterior end detaches itself from the substratum when adhesion is reduced, and, owing to surface tension, endeavours to round itself off. The extension of the anterior end takes place, according to BERTHOLD, with a certain amount of energy, it is *pushed out*, not *pulled out* and the material required for this extension can be provided only out of such parts as lie behind. 'Thus there arises a suction action of the centrally directed current. As far as the anterior margin is concerned, the movement is fan-shaped, because the extension is more vigorous in the centre than it is at the sides.' Another factor of moment to be taken account of is the pressure from behind, associated with the efforts on the part of the posterior margin to round itself off.

BÜTSCHLI advances first of all certain physical objections to this theory. He shows that QUINCKE'S views as to extension, on which BERTHOLD bases

his views, are not quite correct ; he denies the existence of any close adhesion of the advancing margin of *Amoeba* to the substratum, and shows that BERTHOLD'S hypothesis necessitates internal currents in the plasma which must run in the exactly opposite direction to that observed. For this and other reasons BÜTSCHLI holds that BERTHOLD'S theory cannot be accepted, and proceeds to replace it with one of his own. According to BÜTSCHLI amoeboid movement resembles that seen in *emulsions*, as may be seen when one side of an oil drop comes in contact with a soap solution. An oil-soap emulsion suitable for the purpose may be obtained by triturating thick olive oil with potassium carbonate and adding water to the mixture. The soap originally dissolved in the oil passes rapidly into the water, which in turn diffuses into the oil, and the watery soap solution divides up into particles, giving the appearance of minute vacuoles in the oily ground substance. According to BÜTSCHLI'S observations, such a foam shows a strong analogy to protoplasm, which also, according to this author, generally, exhibits a frothy appearance (compare FISCHER, 1901). When some of the vacuoles in such a foam burst unilaterally, the oil at that place becomes covered over with a soap layer and the same conditions arise as when a homogeneous oil drop surrounded by water is allowed to come in contact with a soap solution on one side (Fig. 169). Under these circumstances, the drop as a whole exhibits a progressive movement, and currents are set up in its interior which recall very vividly those seen in *Amoeba*.

The explanation given by BÜTSCHLI of this phenomenon, cannot be discussed in detail here ; we will only note that, in consequence of the lowering of the surface tension at the point of contact with the soap, a disturbance is set up in the equilibrium in surface tension previously existing. One important point in BÜTSCHLI'S explanation must be drawn attention to, viz. that the strongest currents occur immediately on the surface of the oil drop (indicated by the larger arrows in the figure), and that, owing to these, corresponding currents are set up in the surrounding water. BÜTSCHLI has drawn our attention to another point in amoeboid movement, viz. that currents in that case in the water are absent or run in the opposite direction, and he himself rightly concludes that his theory cannot be entirely correct in all its details.

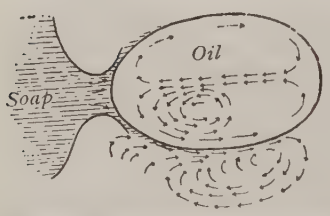


Fig. 169. Oil drop in contact with soap solution. The arrows indicate currents. After BÜTSCHLI (1892).

From another point of view also important objections may be raised to the *foundation* on which both BERTHOLD'S and BÜTSCHLI'S theories are based ; these we may just glance at, without mentioning the special difficulties which arise in comparing the oily foam with protoplasm. PFEFFER (1890) has shown that protoplasm enclosed in a cell-wall is generally in a liquid condition, but that in the plasmodia of Myxomycetes a very obvious cohesion occurs in the passive external layer. Stout strands of *Chondrioderma* may be subjected to a weight of 60 mg. per sq. mm., but these strands recoil to their original length on removal of the weight ; no permanent stretching takes place. Since, obviously, the external passive layer has to withstand this pull practically by itself, PFEFFER calculated that its tensile strength amounts to 300 mg. per sq. mm. When we remember that, in order to tear asunder a lead filament of similar transverse area, a weight of about 2 kg. is necessary, we see that the protoplasm of the Myxomycetes must be a very delicate substance. All the same the cohesive force thus demonstrated proves to us that we are not dealing with a genuine liquid. The cohesion in the peripheral regions is further shown by PFEFFER'S (1890) observation that vacuoles, when carried by the current through narrow channels in the plasmodium, become deformed in consequence.

This cohesion, which is at least noticeable in the surface layer, renders it questionable whether we ought to regard it as *liquid* at all, and whether we may therefore refer alterations in its form to surface tension only. The assumption of an alternation in the condition of the protoplasm from a semi-solid to a liquid state expresses most accurately our present knowledge of the subject. If amoeboid movement is chiefly occasioned, as we are bound to believe, by surface tensions, it must be pointed out at the same time that these tensions are doubtless initiated not by the *environment* but by the protoplasm itself. When the medium in which a plasmodium lies is made perfectly homogeneous, movements still go on in the plasma, and, conversely, a quiescent plasmodium remains unchanged even when alterations in the medium are effected which are calculated to modify its surface tension very greatly (PFEFFER, 1890, p. 275).

As in the case of growth phenomena, so also in locomotory movements, the environment plays a great part [compare EWART, 1903]. Many of these external factors are the *essential formal conditions*, without which locomotion cannot take place. These, or other factors, influence the *direction* of the movement, so that we may divide locomotory phenomena into *autonomous* and *induced*.

The presence of a certain amount of water is one of the most important of the formal conditions of locomotion, for it must be at once apparent that water often acts as the medium in which the movement is carried out. Furthermore, the protoplasm must also itself contain a certain amount of water of imbibition in order that streaming or ciliary motion may take place. Rotation and circulation, it is true, do not cease at once when the cell is plasmolysed, but the resting condition of the peripheral layers may be observed with special clearness in such plasmolysed cells. Ciliary movement also still continues in plasmolysed Bacteria, but if a 5 per cent. to 10 per cent. solution of potassium nitrate be employed to bring about plasmolysis a rigor sets in, which has been termed by A. FISCHER (1894, p. 75) ' drought-rigor ', and which disappears when the water is again replaced. Similar rigor phenomena have been observed by FISCHER in flagella, when these are treated with certain substances, e.g. acids, or when there is a deficiency in nutrients ; narcotics such as ether, as might be expected, produce similar results. Similarly, observations on amoeboid movement show that it, too, ceases when the protoplasm is affected by narcotics, weak ammonia, &c.

Among all the substances which influence movements the action of oxygen is perhaps the most interesting. In many cases the presence of oxygen is an absolutely essential condition for the performance of such movements, but that applies only to aerobic organisms. The anaerobes referred to above cease to move in presence of traces of oxygen, while facultative anaerobes exhibit movements of very varying intensity when oxygen is withdrawn. There is thus no essential interdependence between movement and growth, for some facultative anaerobic Bacteria, according to RITTER (1898), grow very well without oxygen, forming flagella which, however, move only when oxygen is present. Other facultative anaerobes move, for a time at least, *without* oxygen and, if well nourished, their capacity for movement is maintained for a much longer period than if starved. Doubtless the energy required for this movement arises from intra-molecular respiration, for the continuance of which the presence of *sugar* is necessary. According to CELAKOWSKI (1898), *Pelomyxa* continues to move for 72 hours in absence of oxygen, *Oscillaria* for 24 hours, *Chara* for 18 hours, and *Elodea* for 1–4 hours, while the protoplasmic movements in the staminal hairs of *Tradescantia* come to a standstill at once when oxygen is withdrawn. KÜHNE's researches (1898) on Characeae prove that even closely related organisms behave very differently in this respect, for some species continue to exhibit movements, employing intra-molecular respiration, only for hours, others for weeks. A few organisms, e.g. the chromogenic Bacteria investigated by EWART (1897), have the special power of fixing oxygen loosely and making use of this reserve

when oxygen is absent from the environment. Whether or not organisms like *Thiothrix* must be added to this category appears doubtful (WILLE, 1902).

Since the dependence of movement on oxygen is so variable, it is needless for us to attempt the determination of the limits of partial pressure of oxygen permissive of movements in relation to individual organisms; it is manifest at once that each organism must have an optimum, a maximum, and minimum (CLARK, 1888).

The influence of temperature is very striking and many estimates have been made on the subject. The rate of rotation under different temperatures has been accurately studied more especially. The following table, based on the observations of VELTEN on the leaves of *Vallisneria* and condensed by SCHÄFER (1898) will illustrate this :—

1°	5°	10°	15°	20°	25°	30°	31°	32°	33°	34°	35°
0·02	0·06	0·12	0·20	0·26	0·32	0·42	0·43	0·40	0·30	0·17	0·11

It may be clearly seen that the rate of movement increases at first rapidly and then more slowly, until the optimum is reached at 31°, and that, thereafter, it as rapidly falls; above 35° C. heat rigor sets in. The minimum does not always, as in this case, lie somewhere near zero; in many terrestrial plants it obviously lies much higher. The optima and maxima also may be considerably higher, e.g. 40° and 50°, or even above these temperatures (HAUPTFLEISCH, 1892). The phenomena in this respect resemble those of growth, so that we need not pursue the matter further. Similar results have been obtained from the study of the motile swarmspores.

As far as light is concerned, it may be briefly said that its influence on locomotion is indirect only, inasmuch as many organs develop very imperfectly or not at all in the dark and hence, naturally, protoplasmic movement does not take place in them. Further we know that intense sunlight is fatal both to ciliary and protoplasmic movement. If, however, swarmspores or cells exhibiting streaming protoplasm, previously exposed to diffuse light, are placed in the dark, the movements do not appear to suffer any change.

Recently JOSING (1901) has made some remarkable observations on this point. Protoplasmic streaming ceases at once in the dark when the cells are treated with ether or chloroform, with carbon-dioxide or non-volatile organic acids, or salts, but the movements continue in presence of these substances if the cells be illuminated. The same author has recorded additional observations as to the action of ether not less remarkable, but he was unable to give a causal explanation of them.

Still less comprehensible is the well-established fact that protoplasmic streaming may arise in cells which have been separated from the plant though they show no such movements when incorporated in it (KELLER, 1892). [Compare KRETSCHMAR, 1903.] In other cases an already existing protoplasmic movement may be accelerated merely by wounding the cells. It is equally well established that movement may occur in many cases where no injury whatever has been inflicted (HAUPTFLEISCH, 1892). DE VRIES (1885) has pointed out that this streaming effectively aids in establishing a uniform distribution of materials in the cell, and that, too, much more rapidly than by simple diffusion, so facilitating the circulation of nutrients, &c., in the plant. Since, however, protoplasmic movement is not so widespread in the uninjured plant as DE VRIES assumed, obviously such a circulation must be effected without the aid of protoplasmic movement. The further services rendered by streaming, such as the shifting of the position of chloroplasts, we shall treat of in our next lecture. It is impossible at present to pronounce an opinion as to whether the movements which follow after or are accelerated by wounding are to be considered as purposeful

reactions or not. The use of locomotion in free organisms is obvious on the face of it ; where a fixed plant strives to attain a suitable environment and to escape an injurious one by curvatures, free organisms do so by changing their habitat. They frequently attain their object precisely in the same way as in the higher plants, their direction of movement being determined by external factors (Lecture XLIII). Amoeboid movements in protoplasts enable these organisms to absorb solid bodies, which they envelop, and so in their case this movement may be regarded as of importance in nutrition.

Bibliography to Lecture XLII.

Aderhold. 1888. Jen. Zeitschr. f. Naturw. 22, 310.
De Bary. 1864. Die Mycetozoen. 2nd ed. Leipzig.
Berthold. 1886. Studien über Protoplasmamechanik. Leipzig.
Bütschli. 1892. Unters. über die mikrosk. Schäume. Leipzig.
Celakowski. 1898. Bull. de l'Acad. d. sc. de Bohême.
Clark. 1888. Ber. d. bot. Gesell. 6, 277.
Correns. 1897. Ber. d. bot. Gesell. 15, 139.
Engelmann. 1879. Hermann's Handb. d. Physiologie, 1. Leipzig.
Ewart. 1897. Journal of the Linn. Soc. Botany, 33, 123.
[Ewart. 1903. On Protoplasmic streaming. Clarendon Press, Oxford.]
Fischer, A. 1894. Jahrb. f. wiss. Bot. 27, 1.
Fischer, A. 1901. Archiv f. Entwicklungsmechanik, 13, 1.
Hauptfleisch. 1892. Jahrb. f. wiss. Bot. 24, 173.
Hofmeister. 1867. Die Lehre v. d. Pflanzenzelle. Leipzig.
Jensen. 1902. Ascher-Spiro, Ergebnisse d. Physiologie, 1. Wiesbaden.
Josing. 1901. Jahrb. f. wiss. Bot. 36, 197.
Keller. 1890. Ueber Protoplasmaströmung. Diss. Zürich.
[Kretschmar. 1903. Jahrb. f. wiss. Bot. 39, 273.]
Kühne. 1898. Zeitschr. f. Biol. 36, 425.
Lauterborn. 1896. Unters. üb. Bau, Kernteilung u. Beweg. d. Diatomeen. Leipzig.
Müller, O. 1897. Ber. d. bot. Gesell. 15, 70.
Nägeli. 1860. Beitr. z. wiss. Botanik, 2. Heft, p. 96. Leipzig.
Pfeffer. 1884. Unters. bot. Inst. Tübingen, 1, 363.
Pfeffer. 1890. Pasmahaut (Abh. Kgl. Ges. d. Wiss. Leipzig, 16, 185).
Quincke. 1888. Annalen d. Physik, N. F. 35.
Ritter. 1899. Flora, 86, 329.
Schäfer. 1898. Flora, 85, 135.
Schütt. 1899. Jahrb. f. wiss. Bot. 33, 594.
Stahl. 1880. Bot. Ztg. 38, 393.
Strasburger. 1878. Wirkg. des Lichtes u. d. Wärme auf Schwärmsporen. Jena.
Verworn. 1901. Allg. Physiologie. 3rd ed. Jena.
De Vries. 1885. Bot. Ztg. 43, 1.
Wille. 1902. Biol. Centrbl. 22, 257.

LECTURE XLIII

LOCOMOTORY DIRECTIVE MOVEMENTS

In treating of locomotory directive movements we will confine our attention at first exclusively to non-fixed free-moving organisms (Flagellata, Bacteria, Myxomycetes) and consider protoplasts enveloped in cell-walls later. Just as the directive movements in fixed plants have been spoken of under the general term of 'tropisms', so the word 'taxis' has been employed to indicate locomotory directive phenomena ; thus we speak of geotaxis, phototaxis, chemotaxis, &c., according as the direction of the movement is induced by gravity, light,

chemical substances, &c. Among these varied phenomena those of chemotaxis have at present been undoubtedly studied most thoroughly (PFEFFER, 1884 and 1888), and hence it will be most appropriate to begin with them.

One special example of chemotaxis, viz. aerotaxis, has long been known, and we have already become acquainted with the directing influence of small amounts of oxygen on motile Bacteria in discussing the elimination of oxygen by green plants during carbon-dioxide assimilation. Bacteria react to many other substances as well as to oxygen, seeking, so to speak, an optimum concentration of them and retreating from them when their concentrations are too high or too low. Chemotactic movements are thus often purposeful, since they are the means of bringing the organism under optimum vital conditions, but reactions which are apparently purposeless are not unknown, reactions, for example, to substances which the organism is not in the habit of encountering in nature, and to which it is unable to adapt itself. ROTHERT (1901, p. 382) has observed a case of this sort, where two Bacteria were attracted in a remarkable manner by ether, a substance which could only be injurious to them. Again, note must be taken of the fact that, as PFEFFER has observed, many chemotactic organisms are often unconscious of the presence of such poisons as corrosive sublimate or strychnine nitrate, while they run away from such injurious agents as acids, alkalis, &c. Such exceptional behaviour, naturally, does not enter into the question of the biological significance of chemotaxis, which we must assume has always to do with the attainment of optimum environmental conditions. Chemotaxis, however, fulfils a far more special rôle than this in relation to the sexual cells of differentiated lower organisms and of higher plants also. The marked chemotaxis of the male cells leads them to seek the female cell, and fertilization is secured by the attraction exerted on the sperm by certain substances present in the ovum or excreted by it or by parts in its immediate neighbourhood. It had been long suspected that the sperm did not reach the ovum merely by chance but by the attractive influence of certain substances, but exact proof of this was first afforded by PFEFFER (1884), who showed that it was probable that, in the case of the *ferns*, *malic acid* excreted from the *archegonium* was the attractive agent in bringing the sperm into its vicinity.

If antherozoids of ferns be observed under the microscope in a drop of water, they will be seen to move generally in straight lines; whenever they come into the neighbourhood of an archegonium, however, they twist sharply round, so as to direct their anterior ends towards the mouth of the archegonium; they thus rapidly approach it, enter its neck and fuse with the ovum in the interior. In his experiments PFEFFER replaced the neck of the archegonium by a capillary glass tube of about 0·1 mm. bore, which he filled with various substances, placing it at the side of the cover-glass. When the tube was filled with a 0·01–0·5 per cent. solution of malic acid, which was neutralized by appropriate means, PFEFFER found that the sperms rushed towards it and entered it in great numbers. He estimated that 60 sperms had managed to enter such a tube in 30 seconds and 600 of them in 5 minutes. If the movement of the sperms be retarded by using a weak solution of gum it may be clearly seen that the sperms curve round sharply the moment they come within the sphere of influence of the malic acid diffusing out of the tube, and place their long axes parallel with the course of the diffusion current. Without any acceleration of their movements they then steer their way towards the more concentrated solution, straight for the opening of the capillary tube. Since the sperms *distribute themselves equally* in a homogeneous solution of malic acid, just as they do in water, we are bound to regard the unequal distribution of the acid as the directive stimulus. The fact that numerous other substances have no power to induce such directive movements, however, leads us to the conclusion that the diffusion *current*, as such, is not the actual stimulus.

Taking into account the facts we have already learned as to stimuli, we are led in the present instance to seek for the liminal intensity of concentration of malic acid which will give an obvious attractive result. In PFEFFER's experiments that concentration was found to be 0·001 per cent.; still weaker solutions had only a casual effect. As might be expected, the liminal value altered with the age of the organism, just as in the case of other factors, e. g. temperature (VÖGLER, 1891). In fact, extraordinarily minute quantities of various substances may operate as chemical stimuli. In one of PFEFFER'S experiments the tube contained not more than 0·0000000284 mg. of malic acid, and of that naturally only the minutest fraction could come in contact with a single sperm. Such an amount is, however, by no means so insignificant when the weight of the sperm is taken into account; still if the weight of the sperm be estimated at about 0·00000025 mg. it is only ten times as great as that of the malic acid which was used. Quite as minute quantities of other chemicals are able to induce reactions, for DARWIN (1876, p. 246) found that 0·00000328 mg. of ammonium phosphate excited a response in *Drosera*, and ENGELMANN (1883) showed that a trillionth part of a milligram of oxygen could act as a stimulus to Bacteria.

It has already been pointed out that fern antherozoids distribute themselves uniformly in a homogeneous solution of malic acid, but it would be quite incorrect to suppose that the sperms were not stimulated by such a solution; it renders them, as a matter of fact, less sensitive to a unilateral action of the acid and the liminal intensity of the stimulus is higher for sperms swimming in such solution than for those swimming in pure water. Moreover, the increase in the liminal intensity is quite proportional to the concentration of the solution in which they lie. In water 0·001 per cent. of malic acid is required to induce a stimulus.

In 0·0005 %	malic acid,	0·0015 %	is required	to effect	a stimulus
„ 0·001 %	„	0·03 %	„	„	„
„ 0·01 %	„	0·3 %	„	„	„
„ 0·05 %	„	1·5 %	„	„	„

It will be seen that the liquid in the capillary tube must always be 30 times as concentrated as the culture liquid, and hence the absolute difference in concentration necessary to induce a response is much greater when the culture solution is concentrated than when it is weak. We have already become acquainted with the law which governs the relation of sensation to stimulation, a law which was first established with reference to certain human sensitivities and is known as WEBER'S law or the law of psychophysics. So far as regards our own special sensitiveness to weight, for instance, a weight of 1 mg. must be increased $\frac{1}{3}$, a weight of 10 mg. must be increased $\frac{10}{3}$ before we can appreciate a difference between them. This law, it would appear, is of very general, but by no means universal, application. It is limited to certain concentrations, for instance, in the case of fern antherozoids, and does *not* apply to very high or very low concentrations. A capillary tube containing a 0·0003 per cent. solution of malic acid can attract sperms swimming in a 0·00001 per cent. solution, and yet, as we have seen, this solution, after diffusing into water, is unable to act as a stimulus on the sperm; on the other hand, PFEFFER was unable to attract sperms floating in a 0·04 per cent. solution by concentrations either 30, 40, or 50 times as great. In the last mentioned experiment PFEFFER found that the sperms were quite indifferent to a 3–4½ per cent. solution. No end was to be gained by raising the concentration higher than that, for 5 per cent. solutions were found to act quite obviously in a repellent manner. Of this phenomena we shall speak later on.

According to PFEFFER'S experiments, fern antherozoids react only to malic acid and its salts as also to maleic acid (which does not occur in nature), but not to its stereoisomer fumaric acid. The reaction is so specific that antherozoids

it was thought might be employed for determining the presence of malic acid. More recent investigations (BULLER, 1900) have certainly introduced important limitations. Not only malic acid but also a large number of organic and inorganic salts operate attractively, but to all the non-dissociating bodies, such as carbohydrates, glycerine, alcohol, asparagin, the sperms are quite indifferent. The attractive power of the salt is manifested to the best advantage generally when it is in a solution isosmotic with a 0·1 G.M. of potassium nitrate; 1 G.M. for the most part is injurious and 0·01 G.M. is only rarely effective. Malates, on the other hand, act in much weaker concentrations, from 0·01 G.M., down to 0·0001 G.M., and free malic acid from 0·001 G.M. to 0·0001 G.M. Since it is in the highest degree improbable that solutions as concentrated as these are discharged from the archegonium (that is to say equal to a 1 per cent. solution of potassium nitrate, or of a 1·24 per cent. solution of potassium oxalate), it is very likely, but not absolutely certain, that the antherozoids of ferns are attracted by malic acid in the ovum itself, where it probably exists, as PFEFFER has given us reason for believing, not as free malic acid but as a salt of that acid.

As already remarked, there are also substances which act repulsively on antherozoids. If the repulsion is brought about by high concentrations, as in the case of malates, we have to deal rather with an *osmotic* than a *chemical* influence, and hence we may draw a comparison between *osmotaxis* and *chemotaxis* (compare p. 547). Many substances, however, such as alcohol, acids, alkalis, &c., doubtless repel owing to their chemical peculiarities, and, when they do operate, they always do so repulsively only. Other substances, such as free malic acid, attract when in weak concentrations (0·001 G.M.) and repel when in somewhat stronger concentration (0·1–0·01 G.M.). It is not yet clear whether the individual ions operate in a different way, as BULLER thinks.

The antherozoids of *Selaginella* react to malic acid exactly in the same way as do those of ferns, but the specific attractive stimulant has not as yet been determined in the higher Pteridophyta; although such a body is doubtless present there also. [We now know that in all the Pteridophyta the chemotactic stimulant is malic acid (SHIBATA, 1905). Still, interesting differences among these have been discovered, for the sperms of *Equisetum* are sensitive to malic acid *only* (SHIBATA, 1905 c), while those of the Filicinae (SHIBATA, 1905 b) respond to maleic acid as well, but not to fumaric acid; the antherozoids of *Isoetes*, on the contrary, are sensitive to fumaric but not to maleic acid.] Among Bryophyta we know as yet of such attractive chemicals only in the mosses. The remarkable thing in this case is that the attractive substance appears to be cane sugar, a non-dissociating compound. [LIDFORS (1905) affirms that proteids are the chemotactic agents in *Marchantia*.] The antherozoids of mosses are in no sense inferior, so far as sensitivity is concerned, to those of ferns, for the liminal stimulus intensity was determined by PFEFFER in *Funaria* as a 0·001 per cent. for sugar solution.

Chemotactic movements have also been recognized in Bacteria, Flagellata, and in the swarmspores of *Saprolegnia* (PFEFFER, 1888; STANGE, 1890), as the result of the action of various, but not all, materials nutrient to these organisms. Among inorganic bodies, potassium salts and phosphates are effective, among organic substances, peptone and asparagin but not glycerine. At the beginning of this lecture we drew attention to the fact that oxygen had also a chemotactic effect; we may now add that it acts in a strongly repulsive manner to certain anaerobes, although, it must be remembered, other bodies also act in the same way. Many specific differences may be noticed in this relation; certain sulphur-Bacteria, for instance, are attracted by sulphuretted hydrogen, a substance which, under ordinary circumstances, has never that effect on ordinary motile cells (MIYOSHI, 1897). WEBER'S law, as might indeed have been expected, has been found to apply to certain other cases also. There

would have been for all that no need for us to enter into any further discussion of chemotaxis in such organisms, if ROTHERT (1901) had not recently drawn attention to a feature which had escaped previous observers.

Observation of the slow movements of large Bacteria (such as *B. solmsii*) in the vicinity of a capillary tube filled with meat extract shows that although the Bacteria aggregate round it, they exhibit *no alteration in the direction of their movement* when they reach the zone of diffusion; on the contrary, they often pass close to the opening of the tube, maintaining their original direction of movement, and not being apparently stimulated to enter it. At a certain distance from the mouth of the tube they suddenly stop and swim *backwards* (posterior end foremost). Once more they pass the opening of the tube, unaffected by its contents, and again halt at the same distance from it as in the first instance, and again proceed to carry out a forward movement. They are to be met with in a definite zone opposite the centre of the opening of the tube, but their presence there is obviously purely accidental, and the effect of the stimulus is not one of *attraction* but of *repulsion*, induced by the *transition to lower concentrations*, the *response* consisting, not, as in fern antherozoids, in a *reversal* of the anterior end, and in a consequent change in the direction of the movement, but in *locomotion backwards*. After more careful investigation, ROTHERT found that there were greater differences between these two kinds of response than might at first sight have been imagined, for they have only one feature in common, viz. the nature of the stimulant (a chemical compound), while not only the *response* but also the *motive cause* of the *stimulus* and the *sensitivity* as well is different. It is advisable, therefore, that these two movements should be known by different names, and hence ROTHERT describes the movement where the body of the organism is inverted as *strophic chemotaxis*, and that where the organism swims backwards as *apobatic chemotaxis*.

Wherein then lies the difference in the motive cause of the stimulus in these two series of phenomena? PFEFFER and ROTHERT look upon the strophic chemotaxis as due to *differential distribution of the stimulant* on different sides of the plant, as in the case of chemotropism, heliotropism, &c.; in other words, the organism *measures* and *compares* the intensity of the stimulant as effecting different parts of its outer surface. It is impossible, however, that a fern antherozoid can appreciate the inequality of the stimulus on *opposite sides*, since, owing to its rotation on its own axis, any unilateral influence of the stimulant is excluded, just as when a higher plant is rotated on a klinostat. (This criticism, which has not as yet been published, has been communicated to me by OLTMANNS.) The organism must also be able to compare the intensity of the stimulant at its anterior and posterior ends, and, on the analogy of a *dorsiventral* body, must not be in a state of equilibrium, when both ends are subjected to the same intensity of stimulus, but only when the intensity of the stimulus is greater at the anterior end; this will be the case at least as long as positive chemotactic movement follows, in negative chemotaxis the inverse relations hold good.

In apobatic chemotaxis it is possible that the motive cause of the stimulus lies at least in differential concentration of the stimulant at the two poles of the organism, but it is more probable that we have here to do rather with differences in *time* than differences in *place*, and that response occurs when the organism has remained for a definite length of time in a solution of the stimulant less concentrated than that in which it was a short time previously. A homogeneous solution, therefore, must in this case also act as a stimulus, and the bacterium will move backwards if it be transferred from a 10 per cent. solution of meat extract to one of 5 per cent. JENNINGS (1897 and onwards) has, as a matter of fact, shown this to be the case in motile Infusoria (*Paramoecium*), but Bacteria present experimental difficulties too serious for accurate investigation.

This difference in *sensitivity* between apobatic and strophic organisms, discovered by ROTHERT, does not surprise us, since we have already met with similar phenomena in studying the movements of organs in fixed plants. Obviously, the sensitivity of strophically reacting organisms corresponds exactly to that associated with tropisms (in the true sense of the word), while apobatic reactions resemble nastic movements (heat curvatures of tendrils, sleep movements, &c.).

It is to be hoped that ROTHERT'S observations on the difference between apobatic and strophic chemotaxis may soon lead to detailed experimental investigations on the subject; the field for such inquiry is a wide one, and there are still many related problems which are very obscure and of which we can present no explanation here. There is only one point to which we may draw attention, viz. the difference between positive and negative taxis, for in this respect also strophically and apobatically reacting organisms are not alike. So far as strophic organisms are concerned the difference between positive and negative taxis lies only in the *reaction*, the motive cause of the stimulus is the same in both cases and is due to the diffusion of the stimulant into the water. The case is different with apobatic organisms, where the reaction is always the same (a retreating movement); whether a positive or negative taxis takes place, depends only on the motive cause, being positive when the concentration of the stimulant decreases and negative when it increases. The position of the optimum of the stimulant probably determines whether the decrease or the increase in concentration will induce stimulation. Strophic organisms actively seek this optimum, for they turn their bodies sometimes in one direction, sometimes in another, towards it; apobatic organisms, on the other hand, are sensitive, not to the *approach* to but only to the *withdrawal* from the optimum and, in the latter case, retreat from it. It is not impossible that the same organism may exhibit both types of taxis at the same time. [This has been shown to be the case in the sperms of *Isoetes* by SHIBATA (1905 b), and it must be true of fern antherozoids also.]

The existence of the optimum is especially strikingly manifested if the concentrations in any *one* preparation are greatly diversified, for then the motile organisms congregate at a definite place where the optimum concentration prevails. Aggregations such as these are shown by certain Bacteria (*Spirillum*: ENGELMANN, 1881; BEIJERINCK, 1893; *Beggiatoa*: WINOGRADSKY, 1887), which seek regions where there is low oxygen tension and which are negatively aerotactic to high tensions and positively aerotactic to low tensions. Many instances of aggregation of motile organisms at definite *distances* from the mouth of the capillary tube have been demonstrated by PFEFFER and others (ROTHERT, 1901, p. 402). PFEFFER draws special attention to the case of *Spirillum undula*, which may frequently show both positive and negative response to the same stimulant, and, in so far as the action of this stimulant is purely chemical, the difference in the results can be due naturally only to *differences* in concentration. If, however, a *definite* concentration can act at the same time both attractively *and* repulsively, negative osmotaxis must be allowed to be always operating in addition to positive chemotaxis. The fact that *not every stimulant induces positive and negative chemotaxis* leads us to conclude that the optimum for many substances is close to zero, while in the case of others it is very high; in the former case we observe that the stimulant has always a repulsive influence, but, in the latter case, the organisms are always attracted or are indifferent.

We have hitherto spoken of chemotaxis in general and considered the directive influence both of substances in solution and of gases. The question we have now to answer is whether a *gaseous* stimulus operates in the same way as a solution of a *solid body* and whether the sensitivity to different gases

or solutions depends on like or unlike alterations in the protoplasm. When we remember that aerotactic organisms often exhibit no chemotactic sensitivity, and that fern antherozoids are not aerotactic, we must obviously regard aerotaxis and chemotaxis as perfectly distinct sensitivities. Similarly, organisms which are sensitive, for instance, to potassium salts are not necessarily sensitive to oxygen. In short, it is probable that we must assume that there are as many types of chemotaxis as there are chemical substances, or groups of substances, distinguished by the organism. ROTHERT (1901), in fact, has shown, in the case of a species of *Amylobacter*, that chemotaxis in relation to two different substances may indicate a difference in sensitivity in the organism, for he found that this species was chemotactically sensitive both to ether and to meat extract. If both types of taxis are dependent on the same sensitivity, according to WEBER'S Law, the liminal intensity of the stimulus for unilateral action of meat extract will be raised by a homogeneous solution of ether. That is not the case, however. Further investigations on this subject will lead us, no doubt, to extremely important and interesting results as to the powers possessed by organisms for distinguishing between different chemicals and the limits of these. [SHIBATA (1905 b) has, with the aid of this method, been successful in showing that it is possible to decrease the sensitivity of the antherozoids of *Isoetes* to malic acid by using homogeneous solutions of fumaric acid. The effects produced by these two substances on the perceptive apparatus are the same, in other words, the antherozoids could not distinguish between these two substances. On the other hand, the sensitivity of the antherozoids to salts of the potassium group, e.g. of potassium, rubidium, &c., is quite different (BULLER, 1900; SHIBATA, 1905 b).]

What is the exact nature of the first effect of the chemotactically active body and on what the chemotactic perception depends, is as yet entirely unknown. We are ignorant whether the cilia only are the perceptive organs—as is possible; if so, then, in a strophic reaction, it is obviously due to dissimilar concentrations of the solution affecting opposite sides of the cilia. Since, however, the cilia must by their movements neutralize differences of concentration in the fluid, this view does not appear to us to be correct and we prefer to adhere to the hypothesis already formulated, viz. that it is the difference in concentration, anteriorly and posteriorly, that is appreciated. Further, we are ignorant, whether it is necessary that the stimulant must actually enter the organism before a chemotactica stimulus can be produced, since, as PFEFFER (1888) has shown, it may also operate by contact, i.e by merely striking against the protoplasmatic layer. It is more probable, however, that these bodies enter the cell and induce chemical changes in its interior.

It has been already several times pointed out that the osmotic pressure of the solution acts as a stimulus on motile organisms (*osmotaxis*). Proof of this fact has been advanced by MASSART (1889). If *Spirillum undula* and *Bacterium megatherium* be submitted to the attractive influence of a very dilute (0·0005 G.M.) solution of potassium carbonate placed in a capillary tube, the positive chemotaxis may be counteracted by the addition of various substances, and it would appear that the repulsive effect then depends only on the osmotic pressure of these bodies and not on their chemical constitution. Materials with an isosmotic coefficient = 3, such as ammonium chloride, sodium chloride, potassium chloride, &c., initiate a repulsive reaction when the concentration = 0·07 G.M., while substances with 4 as their coefficient induce it at a concentration of 0·05 G.M. to 0·06 G.M. Exceptions are undoubtedly known, but these may be readily explained. Since, e.g., potassium and sodium oxalates or potassium cyanide act repulsively at all concentrations which have been experimented with, the action is not osmotic but chemical. The same result takes place when certain good nutrients act as attractive agents, even when in a high state of concentration,

yet the absence of any repulsive reaction in the case of such substances as glycerine or urea may be due to their known power of penetrating the protoplasm rapidly. An osmotactic effect is absolutely correlated with the impermeability of the protoplasm to the substance under consideration, while its entry is perhaps essential to a chemotactic response. We shall not go far wrong if we look upon the *withdrawal of water* as the main agent in inducing perception in osmotaxis.

Weber's law applies to osmotaxis almost as well as to chemotaxis. In experiments with *Spirillum undula* it may be clearly shown that as the osmotic pressure of the culture fluid increases the liminal intensity of the stimulus necessary for inducing osmotactic repulsion rises. Osmotactic repulsion is induced,

in a normal solution by						0·07 G.M. of sodium chloride		
,,	,,	+0·03 G.M. of sodium chloride			by	0·20–0·25	,,	,,
,,	,,	+0·06	,,	,,	,,	0·25–0·30	,,	,,
,,	,,	+0·09	,,	,,	,,	0·40–0·45	,,	,,

In addition to negative osmotaxis there is also a positive osmotaxis in organisms whose natural habitat is a concentrated medium, and to which they are adapted. This has been shown to be the case by Massart (1891 a) in certain marine Bacteria which exhibit positive osmotaxis. The significance of osmotaxis is closely related to that of chemotaxis, for both sensitivities serve to bring the organism under optimum vital conditions or to retain them there. Many lower organisms are known, however, which are positively chemotactic in highly concentrated fluids, and they collapse at once after being placed in them, as a result of osmotic activity ; osmotactic phenomena are not manifested by these forms at all.

Chemotaxis and osmotaxis are also well illustrated in the plasmodia of Myxomycetes. The fundamental facts were first established by De Bary (1864) and Stahl (1884), still a systematic revision of the phenomena from the point of view gained by similar studies on Bacteria and sperms is as yet non-existent. Since in this case we have to deal not only with alterations in directive movements pure and simple under the influence of chemical stimuli, but at the same time with alterations in the form of the plasmodium, we will not discuss the phenomena presented to us further, but merely note that sensitivity in the Myxomycetes is special in character and dependent on their peculiar habit and relation to a solid substratum. The plasmodia are *hydrotactic*, i. e. they seek situations where a certain amount of moisture prevails or avoid dry substrata. Hydrotaxis is probably very closely allied to osmotaxis. In both series of phenomena it is the withdrawal of the water that leads to perception, and perhaps it is immaterial to the organisms whether the withdrawal is brought about by osmotic activity or by transpiration.

Hydrotaxis in Myxomycetes is also known to be closely related to rheotaxis (Jönsson, 1883 ; Stahl, 1884), a fact which may be easily shown by allowing water to flow off from a vertically placed medium, e. g. filter paper, when the plasmodium moves upwards in the opposite direction to the current. Analogy with rheotropism suggests to us that the motive cause of the stimulus is to be sought for in the *mechanical* action of the water, that is to say, in the *impact* of the fluid on the plasmodium. This fact leads us to mention that ' haptotaxis ' (thigmotaxis), a movement induced by contact stimulus, is also to be recognized as occurring among lower organisms.

Light and heat, like the chemical and physical properties of bodies, also induce directive movements in motile plants, i. e. we must also recognize both phototaxis and thermotaxis. We shall not go far wrong in regarding such types of taxis as entirely analogous to chemotaxis in their nature, inasmuch as

they have for their object the placing of the organism in the most favourable relationship to light and to heat. When the intensity of the heat or the light is graduated, the organisms respond, becoming positively photo- and thermo-tactic at infra-optimal intensities and negatively so at supra-optimal intensities.

There are but few data available as to thermotactic phenomena, so that we may dismiss the subject in a sentence. Thermotaxis has been shown to occur in certain Infusoria, Amoebae (VERWORN, 1901, p. 473), and specially in Myxomycetes. In the case of the last-named organisms STAHL (1884) has demonstrated positive thermotaxis by placing one side of a plasmodium of *Fuligo* in water at a temperature of 30° and the other in water of 7°. He believed that at higher temperatures positive thermotaxis would change into negative, and this idea has been confirmed by WORTMANN'S experiments (1885). The optimum temperature for *Fuligo* lies about 36°, above which negative thermotaxis ensues. Whether or not a difference in the temperatures of the two sides of as much as 20° is necessary has not been determined, but it is probable that it need not be quite as much as that.

Phototaxis, and especially the negative form of it, has also been shown to occur in plasmodia, but the phototactic influence of light has been much more thoroughly studied in relation to swarmspores, whose aggregation on the brightest sides of vessels placed in diffuse light has long been known. Phototaxis is best seen in the swarmspores of Algae, but it occurs also in the colourless swarmspores of *Chytridium*, *Polyphagus*, &c.; it is not exhibited, however, by fern antherozoids. STRASBURGER (1878) has also shown that in many cases the phototactic reaction is dependent on the intensity of the light. If a vessel containing swarmspores be placed at a certain distance from a window, it will be seen that as a rule they arrange themselves so that their long axes are parallel with the path of the incident rays, and with their anterior ends facing the light. Further, the spores proceed to make for the most illuminated spot, moving in straight lines. If the vessel be brought gradually closer and closer to the window the light becomes at length so intense as to cause the swarmspores to retreat from it. Obviously a certain optimum intensity exists between these two intensities, the attainment of which explains the phototactic movement. OLTMANNS' (1892) experiments on this subject are very instructive. He placed motile colonies of *Volvox* in light of very varied intensity and noticed that they always strove to place themselves where they would be subjected to light of a definite intensity. Not all the colonies, however, behave precisely in the same manner; the light requirements of each colony, or—as one might say—its disposition with regard to light varies with its developmental condition. The female colonies, in OLTMANNS' experiment, placed themselves under much feebler illumination than the asexual specimens, where they effected movements which were extremely peculiar but as yet inexplicable. External influences play also an important part in this light disposition. When the illumination is continuous and bright, and when the temperature is at the same time raised, the light requirements are greater, and the colonies migrate to where the light is more intense (STRASBURGER, OLTMANNS).

The crowding together of swarmspores at one point inside a vessel exposed to light of gradually changing intensity is to be ascribed to positive and negative phototactic movements, and also to *indifference* to that intensity to which the region where the crowding occurs is exposed. Although in the case of many swarmspores the region of indifference is by no means sharply defined, in other cases it would appear that it is extremely restricted; for STRASBURGER observed positive phototactic swarmspores becoming negatively phototactic quite *rapidly* as the light was increased without remaining for any length of time in the indifferent condition.

As in the case of heliotropism, so in phototaxis, the question frequently

arises whether it is the direction or the intensity of the light that has the greater influence. There can be no doubt that, in general, phototactic movements are carried out in a state of nature so that swarmspores place their long axes parallel with the incident beam, and further we have every reason to believe that the plant aims not at orientating itself in a definite direction to the path of the rays but at placing itself under an optimum light intensity. The only question is whether it is possible that experimental conditions may be arranged under which no light rays pass from the brighter to the darker region of the apparatus in which the experiment is conducted. According to OLTMANNS it is possible (Fig. 170) to arrange that parallel rays of sunlight may fall at right angles on the lateral wall of a glass trough in which the swarmspores are moving; immediately in front of the wall exposed to the light he placed a prism filled with gelatine in which indian ink had been dissolved. Under these circumstances the light rays will fall on the glass trough parallel to each other (as indicated by the arrows), but their intensity will gradually decrease from one end of the trough to the other. If now phototactic organisms are uniformly distributed in the water in the vessel they will all collect together on the illuminated wall only, and there also distribute themselves uniformly. If they seek, however, a definite intensity of illumination they must obviously move at right angles to the direction of the incident ray. The result of this experiment is that we invariably find an aggregation of swarmspores at a point where the light is of definite intensity, viz. the optimum. Several criticisms may be advanced, which tend to shake our confidence in this experiment; first of all, OLTMANNS has not arranged that the sunlight should fall horizontally and at right angles to the prism, but has allowed it to fall on the darkened side wall in its natural direction, and hence the distribution of light intensity and the direction of the rays in the culture vessel have been somewhat overlooked; but even if the experiment be carried out in the way described all difficulties would not thereby be removed. If the light-absorbing prism be quite homogeneous, and if there be an empty space behind it, then certainly our supposition as to the direction of the rays and the distribution of the light is correct, but in the prism itself, in the glass, and finally in the water in which the organisms are distributed as well, we always find reflection of light, and hence the experiment becomes perfectly useless for the purpose intended (TOWLE, 1901) (compare p. 472).

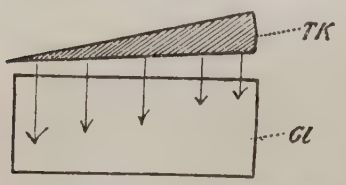


Fig. 170. Plan of OLTMANNS' experimental apparatus. *Gl*, vessel containing experimental material; *TK*, prism filled with indian ink. The arrows indicate the direction in which the light rays fall on the vessel, and their size indicates the intensity of the light.

There are not only physical difficulties to be considered but physiological difficulties as well. ROTHERT'S (1901) observations on strophic and apobatic tactic movements apply naturally not to chemotaxis alone but to all forms of taxis. Apobatic phototaxis we have long been acquainted with; ENGELMANN'S (1882) experiments with *Euglena* are in the highest degree valuable, for they leave no doubt in our minds that these organisms *recoil* when passing from light into darkness. An isolated spot of light with a dark surrounding operates at once upon them; other and earlier experiments carried out by COHN (1852) and FAMINTZIN (1867), and confirmed by STRASBURGER (1878), are unintelligible without the assumption of apobatic phototaxis. In these experiments *Euglena*, *Stephanosphaera*, *Haematococcus*, &c., were placed in shallow dishes which were exposed to direct sunlight, and which were therefore illuminated uniformly; if a narrow plate is then laid transversely across the vessel the motile organisms rapidly assemble in the half-shaded places, leaving the regions of greatest shade and also those on which the direct sunlight falls. It is quite impossible

that swarmspores illuminated on all sides by light of uniform intensity can have any knowledge of the fact that there exists a region at a certain distance from where they happen to be at the moment where the intensity of the light is more adapted to their requirements ; it is only *by chance* that they reach that situation, where they remain on account of apobatic phototaxis. It appears to us in the highest degree probable that *Volvox* also is apobatically phototactic, although at the same time we are not prepared to deny that it is also strophically so. Further investigations must be carried out upon the subject so as to determine in how far light intensity and the direction of the rays affect phototactic movements.

From what we have learnt in regard to heliotropism we have the right to expect that all wave lengths have not the same value in phototaxis ; in fact, experiments designed for this very purpose have shown that the more refrangible rays have obviously much greater phototactic influence than the less refrangible.

As to the *primary* physical or chemical effect of light leading to perception nothing at all is known ; nor is the *region* where light perception occurs sufficiently accurately determined. As in the case of chemotaxis so in the case of strophic phototaxis we must assume that the organism reacts to differential illumination of the anterior and posterior ends, but that in apobatic taxis localization of light perception is possible at the anterior end. It is known that in many swarmspores, &c., there appears in the otherwise colourless anterior ends, a red spot which has been termed the 'eye-spot,' and to which sensitiveness to light has been attributed, but there are certain phototactic swarmspores which possess no such spot, and hence it would appear very improbable that it has any significance in relation to light perception.

There are also certain free-moving organisms which exhibit a directive response to the galvanic current (*galvanotaxis*) ; this has been shown to be true especially of Amoebae and Infusoria, although there is evidence of it also in the higher animals (VERWORN, 1901, 476) ; it is probable that similar phenomena will be discovered in typical plants. Amoebae and Infusoria, general speaking place themselves so that their long axes lie in the direction of the current, creeping or swimming towards the negative pole ; certain Flagellata behave exactly in the contrary way, aggregating round the positive pole. It is very probable that galvanotaxis is not due to a special sensitiveness on the part of the organism to the electric current itself, but rather results from the chemical decomposition which the current gives rise to. According to LOEB and BUDGETT (1897) free alkali arises at the anode end of the organism, and this induces negative chemotactic movements towards the kathode. How it is that some Flagellata move towards the anode remains yet unexplained.

Finally, we have still to speak of *geotaxis*, which has been demonstrated as occurring in Bacteria, Flagellata, &c., by SCHWARZ (1884), ADERHOLD (1888), and MASSART (1891 b). Many organisms, when other attractive forces are excluded, move upwards or, in other words, are negatively geotactic. MASSART found in the case of two species of *Spirillum* which were equally sensitive tonotactically and aerotactically, that the one was positively and the other negatively geotactic. Whether, in the case of geotaxis, we have to do with some sensitivity which may be associated with geotropism appears to us very problematical ; for geotactic *upward* movement cannot bring the organism under conditions where the *influence of gravity* is different from what it was before, while a phototactic movement is capable of placing the organism in other light intensities, just as a chemotactic or osmotactic movement brings the organism into liquids of different concentration.

Still it must be extremely useful to an organism which lives under definite vital conditions to be able to reach more superficial or deeper layers of a fluid

medium. We are led to believe, therefore, that such an organism must possess the power of appreciating *how* deep it is in the medium ; such a sensitiveness cannot arise in any way from the *direct action of gravity* but it may be due to its power of appreciating the *pressure of the liquid* upon it. In fact JENSEN (1893) has endeavoured to refer geotaxis to perception of this pressure, although he was unable to offer exact proof of the truth of his hypothesis.

Tactic movements are not limited only to free organisms ; they occur also in protoplasm enclosed within cell-walls, and especially in certain organs of the cell, such as chloroplasts and nuclei. In the former case we are acquainted with remarkable phototactic movements, in the latter the movements are traumatotactic arising especially after wounding. The movement of chloroplasts may be best studied in *Mesocarpus* ; only one chloroplast, which takes the form of a flat, rectangular band, is present in each of the cylindrical cells of this alga.

In Fig. 171, *I*, the band is shown, in a transverse section of the cell, in the position which it takes up when subjected to light of medium intensity ; the chloroplast behaves itself under these conditions just like a heliotropic leaf placing itself at right angles to the incident ray, and hence, presenting its greatest surface to it. If the intensity of the light increases, however, the chlorophyll plate twists round through an angle of 90° and finally presents its edge to the light (correspondnig to the profile position of the foliage-leaf). Experiments in illustration of this fact may be readily carried out; they have been favourite lecture experiments ever since the phenomenon was first described by STAHL (1880). Nevertheless there are many important details with regard to these movements which have not yet been explained, for instance, is the chlorophyll itself active ? in answer to which it must be admitted that motile organs in connexion with the chloroplast have not yet been discovered. Does it move passively ? How comes it about then that the active protoplasm twists the chlorophyll plate only until it reaches the desired position ? Were it simply the case that the protoplasm lying on the side previously receiving the most light moved away from that side as the intensity of the light increased and passively carried the chloroplast with it, then the whole phenomenon would be perfectly intelligible. But as a matter of fact the protoplasma touching one edge of the chloroplast must perform a movement exactly opposite to that touching the other edge. It is quite inexplicable how it is that after sufficiently long illumination the twisting of the chloroplast is carried out in the dark only to the same extent as it would be if continuously illuminated (LEWIS, 1808). How does the plate know when it has turned through an angle of 90° ?

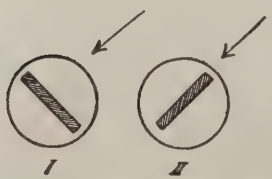


Fig. 171. Diagrammatic transverse section through a cell of *Mesocarpus*. The chloroplast is represented by the shaded band in the middle; the arrows indicate the direction of the light.

The phenomena concerned in the movements of many small chloroplasts in a cell are more easily understood. Here also we meet with a surface and a profile orientation but these arise not by simply twisting the chloroplast in situ but by transferring them to more appropriate situations. The surface orientation is attained when the chloroplasts arrange themselves over the illuminated wall of the cell, the profile position when they lie parallel with the path of the incident ray, and therefore at right angles to the surface of the cell exposed to light. Fig. 172 represents a transverse section of the frond of *Lemna trisulca*, the arrows indicating the path of incidence of the light rays. At *T* the chloroplasts are represented in the superficial position which they assume in diffuse light, at *S* they have taken up the profile position in direct sunlight. It must be noted, however, that a third, or night orientation may be observed in which some of the chloroplasts are in the profile, some in the superficial position, but where

the external walls are always free of chloroplasts. This night position is not found in all plants; in many shade plants and aquatics all the chloroplasts are superficially arranged in darkness. In plants which distinctly prefer bright sunlight, the nocturnal profile position may be assumed on subjecting them to light of relatively slightly diminished intensity. The day profile position also occurs in various plants when subjected to light of varying intensity, an intensity which is feeble in shade-loving plants and high in those which prefer bright sunlight. Even in the cells of the *same* leaf differences manifest themselves, for the cells of the under-side of *Elodea* arrange their chloroplasts in the superficial position before those of the upper side (MOORE, 1887).

The significance of the profile position in bright light is generally intelligible, for this position enables the chloroplasts to arrange themselves in such a manner that they may receive the exact amount of light they require, just as a leaf can by changing its position. The nocturnal profile position has, however, yet to be explained. [It is obvious that in producing the nocturnal position chemotactic movements co-operate, and carbon-dioxide more especially must play a part in determining the position of the chloroplasts, for it must accumulate on the inner and lateral walls of the cells and be less apparent on the outer walls (SENN, 1904).] How the two positions are arrived at in Mesocarpus has already been noted; we have no information, however, as to whether the movements are active or passive, although if they be passive they are more readily intelligible in the present instance.

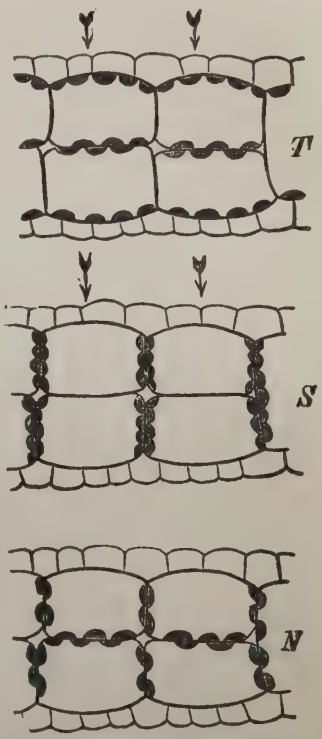


Fig. 172. Transverse sections through the frond of *Lemna trisulca*. *T*, position of chloroplasts in diffuse light; *S*, in bright light; *N*, at night. After STAHL, 1880. From the Bonn Textbook.

It is impossible for us to enter more fully into the discussion of certain other phenomena presented by chloroplasts closely related to those we have discussed, such as the aggregation of the chloroplasts in the angles of the cells under high intensities of light and their *change of form* in palisade cells; we must content ourselves with observing that the external brighter or darker green colour of the plant is often due to changes of position of the chloroplasts.

Passive changes in position of the nucleus may generally be observed whenever the protoplasm shows signs of vigorous movements, and these movements are autonomous in their nature. Induced movements and movements towards a definite region take place after wounding. TANGL (1884) was the first to show that injury to the epidermis of the scales of the onion induced a movement of the nuclei towards the surface which had been wounded; in that region also the protoplasm tended to collect. To NESTLER (1898) belongs the credit of having demonstrated the very general occurrence of traumotaxis, but it is to NĚMEC (1901) that we owe the most thorough investigations on the subject, and especially the determination of the rapidity with which the stimulus due to injury travels and the induced position is replaced by the normal. For all the new facts on this subje t we must refer our readers to NĚMEC'S papers. MIEHE (1901) and KÖRNICKE (1901) have also recorded remarkable observations as to the migration of nuclei. Under certain

not very clearly defined but still anomalous conditions these authors observed the transference of nuclei through apparently intact cell-walls into neighbouring cells after injury had been inflicted. We mention these observations chiefly because we have previously had occasion to refer to the subject. If it can be shown that this transference of nuclei into quite normal cells is a physiological reaction, the fact is one of great importance in many respects. Proof of this is, however, not forthcoming, and it is, on the contrary, very probable that such a transference of nuclei is quite pathological and perhaps passive in its nature.

Bibliography to Lecture XLIII.

ADERHOLD. 1888. Jen. Zeitschr. f. Naturw. 22, 310.
DE BARY. 1864. Die Mycetozoen. 2nd ed. Leipzig.
BEIJERINCK. 1893. Centrbl. Bact. 14.
BULLER. 1900. Annals of Botany, 14, 543.
COHN. 1852. Zeitschr. f. wiss. Zoolog. 4, 111.
DARWIN. 1876. Insektenfressende Pflanzen (CARUS). Stuttgart.
ENGELMANN. 1881. Pflüger's Archiv, 26.
ENGELMANN. 1882. Ibid. 29.
ENGELMANN. 1883. Bot. Ztg. 41, 4.
FAMINTZIN. 1867. Jahrb. f. wiss. Bot. 6, 1.
JENNINGS. 1897 and onwards. Quoted by ROTHERT, 1901.
JENSEN. 1893. Pflüger's Archiv, 53.
JÖNSSON. 1883. Ber. d. bot. Gesell. 1, 512.
LEWIS. 1898. Annals of Botany, 12, 418.
[LIDFORS. 1905. Jahrb. f. wiss. Bot. 41, 65.]
LOEB and BUDGETT. 1897. Pflüger's Archiv, 65.
KÖRNICKE. 1901. Sitzungsber. niederrh. Gesell. Bonn.
MASSART. 1889. Archives de Biologie (van Beneden and Bambeke) 9, 515.
MASSART. 1891 a. Bull. Acad. Belg. 22.
MASSART. 1891 b. Ibid. 22, 158.
MIEHE. 1901. Flora, 88, 105.
MIYOSHI. 1897. Journal of College of Sc. Tokyo, 10, II, 143.
MOORE, SP. LE MARCHANT. 1887. Journal Linn. Soc. 24, 200.
NĚMEC. 1901. Reizleitung u. reizleitende Strukturen. Jena.
NESTLER. 1898. Sitzungsber. Wiener Akad. Math. nat. Kl. 107, I, 706.
OLTMANNS. 1892. Flora, 75, 183.
PFEFFER. 1884. Unters. bot. Inst. Tübingen, 1, 363.
PFEFFER. 1888. Ibid. 2, 582.
ROTHERT. 1901. Flora, 88, 371.
SCHWARZ, FR. 1884. Ber. d. bot. Gesell. 2, 51.
[SENN. 1905. Dunkellage d. Chlorophyllkörner. Verh. d. schweiz. naturf. Gesell. Winterthur.]
[SHIBATA. 1905 a. Bot. Mag. 19, 39.]
[SHIBATA. 1905 b. Jahrb. f. wiss. Bot. 41, 561.]
[SHIBATA. 1906 c. Bot. Mag. 19, 79.]
STAHL. 1880. Bot. Ztg. 38, 297.
STAHL. 1884. Ibid. 42, 145.
STANGE. 1890. Bot. Ztg. 48, 107.
STRASBURGER. 1878. Wirkung des Lichtes u. d. Wärme auf Schwärmsporen. Jena.
TANGL. 1884. Sitzungsber. Wiener Akad. Math. nat. Kl. 90, I, 10.
TOWLE. 1900. Journal of Physiol. 3, 360.
VERWORN. 1901. Allgem. Physiologie. 3rd ed. Jena.
VÖGLER. 1891. Bot. Ztg. 49, 641.
WINOGRADSKY. 1887. Bot. Ztg. 45, 493.
WORTMANN. 1885. Ber. d. bot. Gesell. 3, 117.

INDEX

Absorption by insectivorous plants, 186.
Absorption, in the soil, 92, 93; of light, by chlorophyll, 129; of heat, 398.
Acceleration of growth, in geotropism, 434; in haptotropism, 493; in nyctitropism, 501; by stimuli, 300.
Accumulation of materials in cells, 20.
Acetic acid, Bacteria, 216; as a product of fermentation, 216, 218; oxidation of, 216, 218.
Acids, organic, *see* Organic acids.
Acquired characters, inheritance of, 391.
Acropetal, 274.
Action, physiological, at a distance, 485.
Activities of the organism, 247, 397.
Adaptation, capacity for, 389, 391; hereditary fixation of, 389.
Adaptations, 389; active, 389; to external factors, 389; inheritance of, 391; to concentration, 179; to locality, 253; direct, 254; functional, 251, 331, 391; inheritance of functional, 392; passive, 390; inherited, 254.
Adaptive characters, 386.
Adventitious origin, of growing points, 284; of embryos, 370.
Aerial roots, heliotropism in, 461; growth in, 289.
Aeriferous system, 37.
Aerobionts, 213, 526.
Aerotaxis, 105, 542, 547.
Aerotropism, 484.
After-effect of external factors in geotropism, 437; in adaptations, 390, 392; in periodic phenomena, 343; in periodic movements, 508.
Aggregation of protoplasm in *Drosera*, 498.
Agriculture and nutrient materials, 101.
Air-bubbles, counting of, in assimilation, 104.
Albumins, 140.
Albumoses, 139.
Alcohol, in fermentation, 208; in intramolecular respiration, 203.
Alcoholase, 212.
Alcohols, higher, in fermentation, 214.
Aleurone, 160.
Alinite, 235.
Alkaloids, 4, 176.
Alpine plants, 319.
Alternation of generations, 358.
Aluminium, 86.
Amide-organisms, 181, 241.
Amido-compounds, 4, 139; assimilation of, 143; formation of, 173; as reserves, 163; transformation of, in seedlings, 173; translocation of, 167.
Ammonia, assimilation of, 135; formation of, from urea, 224; formation of, from peptone, 200; oxidation of, 228; occurrence of, in nature, 135; occurrence of, in the soil, 137.
Amoeboid movement, 534.
Amphibious plants, 253.
Amphimixis, 371.
Amylase, 152.
Anaerobes, 213; biological significance of, 215; oxygen requirements of, 215; injurious influence of oxygen on, 215.
Anaesthetics, *see* Narcotics.
Analysis, *see* Composition.
Anaphase in nuclear division, 268.
Aniline dyes, osmosis of, 20.
Anisophylly, 313.
Annual periodicity, *see* Periodicity; annual rings, 350.
Anthers, dehiscence of, 416.
Anticlinal, 280.
Antienzymes, 156.
Apex, 273, 331.
Apical cell, 279; growth, 261.
Apogamy and apospory, 359.
Apposition, *see* Cell-wall, Growth.
Archegonium, 359, 361.
Arginin, 140, 160, 174.
Arrangement of lateral organs, 274.
Articulations of leaves, 454.
Asci, ejection of spores from, 422.
Ash, 77–102; non-essential constituents of, 86; origin of, 77; amount of, 79, 80; dependent on substratum, 79; dependent on transpiration, 79; essential constituents of, 80, 81; nature of, 78.
Asomatophytes, 273.
Asparagin, 143, *see* Amido-compounds; accumulation of, in darkness, 174; influence of, on diastase, 152; as a nutrient, 144, 182.
Assimilation, products of, transformation of, 147; of materials of the ash, 83, 178; of carbon by autotrophic plants, 102, *see* Carbon-dioxide; by heterotrophic plants, 178; of nitrogen, *see* Ammonia, Asparagin, Amido-compounds, Proteid, Nitrogen, Peptone, Nitric Acid; proper, 259; products of, in carbon-dioxide decomposi-

tion, 110; products of, in nitrogen assimilation, 138; amount of products of, 114, 116.
Atavism, 373.
Autonomous movements, 428, 521, 528; induced by internal stimuli, 527; induced by variation, 528; induced by growth, 529.
Autotrophic nutrition, 177.
Autotropism, 448; in geotropic curvature, 432, 448; in haptotropic curvature, 493; in mechanical curvature, 493; in nyctitropic curvature, 507.
Auxanometer, 287.
Averrhoa, autonomous movements in, 528.
Axillary bud, 278.

Bacteria, movements, *see* Natatory movements, Chemotaxis, Aerotaxis, &c.; nutrition of, *see* Heterotrophic plants.
Bacterium radicicola, 237.
Bacteroids, 237.
Base, 273; in regeneration, 330.
Basidiobolus, development of, 248; dependence of, on nutrition, 248.
Beggiatoa, 221; organic nutrients of, 223, 229; oxidation of sulphuretted hydrogen by, 221; oxidation of sulphur by, 222; oxygen requirements of, 222.
Benzol-derivatives as nutrients, 180.
Bilateral, 276.
Bleeding, 50; significance of, 56; conditions of, 53; duration of, 51; pressure in, 52; local, 54; mechanics of, 54; sap, 51.
Bordered pits, 68.
Branches, amputation of, 351; autotropism in, 449; essential angle of, 449; excentric thickening in, 314; plagiotropism of, 449; factors in the direction of growth of, 449; change in, to orthotropy, 449.
Branching, 26; of the leaf, 278; dichotomous and lateral, 274; of the shoot, 278; of the root, 283.
"Budding" in horticulture, 333.
Buds, propagative, 362, 365.
Butyl-alcohol, as a product of fermentation, 214.
Butyric acid, as a product of fermentation, 217, 218.

Caesium, 84.
Calciphilous, 99.
Calciphobous, 99.
Calcium, 84; oxalate, 141.
Callus, origin of, 328; formation of members from, 329.
Cambium, 294, 350.
Cane sugar as a reserve, 162.
Capacity, 341; selective, 20.
Capillarity, in water conduction, 72.
Carbohydrates, 4; as products of assimilation, 115; as respiratory material, 196; as fermentable material, 208; as reserves, 162, 163; relation of, to fats, 158.
Carbon-assimilation, in autotrophic plants, *see* Carbon-dioxide; in heterotrophic plants, 177; products of, 103, 110.
Carbon compounds, nutritive value of, 178, 179.
Carbon-dioxide, assimilation of, *see* Assimilation; dependence of, on external factors, 119, 123, 124; on chlorophyll, 103; on light, 103; absorption of, 118–22; excretion of, by the root, 95; source of, 103; occurrence of, 118, 119; decomposition of, 103, 110.
Cardinal points of temperature, 124, 201, 299, 526.
Carnivorous plants, *see* Insectivorous plants.
Catalytes, 152.
Catasetum, slinging movements in, 426.
Cell aggregates, 272.
Cell-nucleus, 6, 268; movements of, 553.
Cells, rounding off of, 296; mature, 296; structure of, 6; as fundamental units, 258; embryonic, 295; form of, 296; contents of, 297; osmotic characters of, 13; regeneration of, 380; growth of, 258; fusion of, 297; division of, 268.
Cell-sap, 6, 7.
Cell-wall, 297; formation of, 260; stretching of, due to osmotic pressure, 419; lamellation of, 266; growth of, by apposition, 262, 266; cessation in growth of, 267; significance of the nucleus in growth of, 268; osmotic pressure in growth of, 265; growth in thickness of, 265; growth in surface of, 260; intercalary growth in, 261; growth by intussusception of, 262, 264; growth by cap formation in, 263; growth by interpolation of protoplasm in, 266; growth by plastic stretching of, 263; at the apex, 261; reduction of tension in, 421.
Cellular plants, 258.
Cellulose, as a reserve material, 158; dissolution of, by cytase, 158; by Fungi, 183; fermentation of, 218.
Centrifugal force, 430.
Centrosome, 368.
Chain, Jamin's, 71.
Chain of releasing stimuli, 525.
Chemical stimuli, in relation to spores of Fungi and to pollen, 317; influence of, on form, 317, *see also* Chemotropism, Chemotaxis, *Drosera*, Galls, Poisons, *Mimosa*, Tendrils.
Chemotaxis, 541; by malic acid, 542; apobatic, 545; significance of, 542; by gases, 546; negative, 545; positive, 545; stimulative agents in, 542, 545, 547; perception of stimulus in, 547; liminal intensity in, 543; repulsion in, 544; stro-

phic, 545; occurrence of, 542; Weber's law, in relation to, 544.
Chemotropism, 481; by gases, 484; by dissolved bodies, 482; in Fungi, 482; of pollen-tubes, 483; Weber's law in relation to, 483.
Chlorine, 83.
Chloroform, *see* Narcotics.
Chlorophyll, chemistry and physics of, 107; effect of darkness on, 308.
Chloroplast, 6; as the organ for decomposition of carbon-dioxide, 107.
Chlorosis, 85.
Cholesterin, 4; in the plasmatic membrane, 22.
Chromatin, 268.
Chromosomes, 268; formation of, 377; as hereditary agents, 377; number of, 368, 378.
Cilia, as motile organs, 533.
Circulation of protoplasm, 536; of carbon and nitrogen, 243.
Circumnutation, 529.
Citric acid, 197.
Climbing plants, 455.
Closing of flowers, 500.
Clostridium pasteurianum, 233.
Cobalt-paper, 36.
Coefficient, isosmotic, 16; economic, 191.
Cohesion of water, significance of, in water conduction, 65, 72; in dehiscence, 415; in imbibition, 417.
Cold rigor, 300.
Collective species, 385.
Colloidal solutions, 153.
Colonies, 272.
Colouring matters, 4, 176; formation of, in darkness, 308; diosmosis of, 20.
Combustion, chemical and physiological, 205.
Comparison of intensities, 524.
Compass plants, 466.
Compensation, 330.
Composition, chemical, 3, 5.
Concentration, influence of, in osmotic pressure, 15; on growth, 316; as a directive stimulus, 546.
Constructive material, 178.
Contact stimulus, 490; movements in consequence of, 487; organogenetic results of, 314.
Continuity of the embryonic substance, 284.
Contractile layer, 411.
Contractile threads, 268.
Contraction ellipsoid, 410.
Contraction in secondary growth, 295.
Copper, 87, 88.
Cork, 327.
Correlations, 252, 326; demonstration of, 327, 331, 332; quantitative and qualitative, 330; as regulating growth, 252; in movements due to stimuli, 524; causes of, 335; between leaves and buds, 330; between leaves and conductive strands, 330, 335; between buds, 329; between shoots, 330; between parts of the cell, 335.
Corrosion, of starch, 155; by the root, 95.
Corrosive sublimate, 87, 542.
Culture on solid substrata, 80; in aqueous solutions, 80.
Curvature, 406; geotropic, 431; work accomplished in, 437; course of, 432–5; heliotropic, 460–75; in shrivelling, 409; due to turgor and growth, 421; due to weight, 448.
Cuscuta, carbon assimilation in, 188; twining of, 496.
Cuticle, permeability of aerial, 33, 38, 120; permeability of subterranean, 33.
Cyclanthera, ejaculatory movements in, 425.
Cynareae, movements of the stamens in, due to stimulus, 519.
Cytase, 152, 158, 183.
Cytoplasm, 6.

Daily periodicity, 508.
Darkness, *see* Etiolation.
Darkness-rigor, 302, 508.
Darwinian theory, 384.
Day position, 500.
Day sleep, 505.
Death, 351, 362.
Definite branches, 277.
Denitrification, 232.
Descent, theory of, 383.
Deserts, 97.
Desiccation, 34, 318; respiration during, 341.
Desmodium, autonomous movements in, 528.
Destructive metabolism, 207.
Development, 250; of branches, *see* Branches, Growing point; inhibition of, in sexual cells, 371; stimuli inducing, 369.
Dextrin, formed from starch, 150.
Dextrose, *see* Carbohydrates.
Diageotropism, 446.
Diaheliotropism, 464.
Diastase, 149, 152; nature of, 150; coordinate formation of, 183; effect of temperature on, 151; effect of accelerating agents on, 151; effect of poisons on, 151; occurrence of, 149, 164, 183.
Diatomaceae, movements of, 534.
Dichotomy, 274.
Differentiation, 251; internal, 284, 296.
Diffusion, 13; of carbon-dioxide, 120; as an agent in transport of materials, 167; as an agent in directive movements, 481, 542, 547.
Diminution, harmonious, 315.
Directive movements, 428.
Disaccharides, hydrolysis of, 164.
Dissimilation, 190.
Dissociation, 16.

Dissolution of reserves, 147-65.
Division, of the nucleus, 268; of the cell, 269, 270; amount of, 271.
Dorsiventral, 277.
Drosera, protoplasmic aggregation in, 498; movements in, 496; chemical stimuli as affecting, 497; as an insect trap, 185, 496; contact stimulus in, 497; mechanics of curvature in, 497; nastic and tropistic curvature in, 499; direct stimulation in, 498; indirect stimulation in, 498.
Drought rigor, 539.
Dry weight, decrease of, in darkness, 191; increase of, in water cultures, 80, 82.
Duramen, conduction of water in, 62.
Dynamical layers, 412.

Ecballium, ejaculatory movements of, 424.
Egg, development of, inhibition of, 368; development of, stimulation of, 370.
Ejaculatory movements, of *Catasetum*, 426; of ferns, 415; of fruits, 424; of Fungi, 423; of stamens, 425; causes of, 410, 415, 422.
Elastic stretching of the cell-wall, 263.
Electricity, production of, 402.
Electrotropism, 480.
Elements of the ash, 80.
Elongation, 285.
Embryonic substance, 273; continuity of, 284; growth of, 284.
Endosmosis, 13.
Endosperm, 148, 155; depletion of, in absence of embryo, 155; depletion of, effects of oxygen and chloroform on, 156.
Energy, conservation of, 397; forms of, in the plant, 397; sources of, 397; mechanical, 403; due to respiration, 403; due to other processes, 404; transformation of, 2, 397.
Enzymes, 149, 152; hydrolytic, 152; inorganic, 153; catalytic action of, 152; synthetic action of, 154; incomplete reactions by, 153, 154; specific action of, 154; action of, on peroxide of hydrogen, 153; oxidizing, 204; zymotic, 211.
Epinasty, autonomous, 530; induced, 449.
Epithem, 58.
Equimolecular, 16.
Ether, chemotactic influence of, 542; influence of, on assimilation, 195; influence of, on respiration, 195, 202; lowering of sensitivity by, 516; influence of, on the formation of shoots, 345.
Ethereal oils, 4, 176.
Ethyl-alcohol, *see* Alcohol.
Etiolation, by withdrawal of light, 304; significance of, 306; by withdrawal of nitrogen, 315.
Excitation, geotropic, 438; heliotropic, 469; conduction of, 444, 469.
Excreta, 176.
Exosmosis, 14.
Experiment, 2.
Extensibility of the cell-wall, 420, 520.

Factors concerned, in plant form, 339; in plant habit, 255; in a mechanism, 255.
Fats, 4, 158; as products of assimilation, 158; as respiratory material, 200; formation of, from carbohydrates, 175; hydrolysis of, 159; as reserves, 159, 162; transformation of, into carbohydrates, 158.
Felspar, weathering of, 91.
Fermentation, 214; alcoholic, of sugar, 208; significance of, 213; influence of oxygen on, 213; material of, 208; by-products in, 210; products of, 210; relation to respiration of, 212; zymase in, 211; *see* also Butyric acid, Butyl-alcohol, Cellulose, Acetic acid, Pectin, Organic acids.
Ferments, *see* Enzymes.
Fertilization, 354, 358, 361; significance of, 367, 376; of the embryo-sac nucleus, 361, 370.
Flanks, 276.
Flower, 349, 360; formation of the, due to specific materials, 349; on cuttings, 364; causes of, 363, 364; and vegetative growth, 364.
Flowering plants, functions of organs in, 250; differentiation and division of labour in, 250; development of, 250; segmentation of, 250.
Fluctuating variations, 387.
Foliage-leaf and scale-leaf, 349.
Form and material, 256.
Formal conditions, 253, 427, 522, 526–8.
Formaldehyde, as a product of assimilation, 112.
Formation, 258.
Formative stimuli, 298.
Freezing, 300.
Fruits, ejaculatory movements of, 410, 424.
Function, inhibition of, results of, 331; transference of, 331.
Functional adaptation, 251, 331, 392; stimuli, 335.
Fungi, influence of substratum on, 320; nutrition of, *see* Heterotrophic plants.
Fusion in fertilization, 354, 355, 358, 361.

Galls, 320; of *Dryophanta*, 324; due to insects, 321; due to Fungi, 321; of *Spathegaster*, 323; causes of, 324; purpose of, 324.
Galtonian curve, 387.
Galvanotaxis, 551.
Galvanotropism, 481.
Gametes, 355.
Gases. *see* Carbon-dioxide, Oxygen, &c.
Geotaxis, 551.
Geotropism, 429; in dorsiventral structures, 452; excitation in, 439; in pulvinate

leaves, 454; intermittent stimulation in, 438; use of klinostat, 430; Knight's research, 430; correlative influences in, 450; curvature in, *see* Curvature; after-effects in, 437; negative, 431; in orthotropic organs, 429; perception in, 440; in plagiotropic organs, 445; positive, 431; latent period in, 439; protoplasmic movement in, 444; chain of stimuli in, 444; in rhizomes, 446; rest position in, 440, 447; in lateral roots, 447; in lateral branches, 448; statolith theory in, 442; disposition of organs to, 450-2; torsion in, *see* Torsions; in twining plants, *see* Twining plants; in conjunction with heliotropism, 476.
Germination, capacity for, 341; of seeds, translocation of materials in, 147; conditions of, 252.
Glucosides, 4, 176.
Glutamin, *see* Amido-compounds.
Glycerine, diosmosis of, 19; as a product of fermentation, 210; as a nutrient, 179; esters, 4.
Glycogen, 189.
Graft hybrids, 381.
Grafts, 333.
Gravity, its activity cancelled by the klinostat, 429; as a releasing stimulus, 436; influence of, on secondary growth in thickness, 314; on growth in length, 314; on direction, *see* Geotaxis, Geotropism; on symmetry, 313; replaced by centrifugal force, 430; intensity of, 430; direction of 313, 440; liminal intensity of, 439.
Growth, 258; influence of poisons on, 316; of oxygen on, 316; duration of, 294; embryonic, 284; and reproduction, 358; without reproduction, 362; rate of, 293; of branches, *see* Branches; of protoplasm, 259; spasmodic variations in, 292; distribution of, in branches, *see* Branches; of the cell, artificial, 260; of the cell-wall, *see* Cell-wall; movements, 421, 529; capacity for, 300; periodicity in, 284, 295; zones of, 288.

Habitat, 389; adaptation to, 253.
Halophytes, 97, 319.
Haptotropism, 487; in Algae, 499; in *Drosera*, 496; in Fungi, 499; in tendrils, 487.
Harmonic dwarfing, 315.
Harmonious development, 252.
Haustoria, induced by contact, 496.
Heat, production of, 399.
Heating, due to respiration, 400; due to radiation, 43.
Heat-rigor, 300.
Heliotropism, 460; in *Avena*, 469; relation of, to geotropism, 460; in diffuse and direct light, 466; in dorsiventral organs, 464, 466; sensitivity in, 463, 469; excitation in, 469; surface position in, 466; curvature in, *see* Curvature; light intensity in, 463; in nature, 464; negative, 460; in orthotropic organs, 461; in Paniceae, 468; in conjunction with geotropism, 476; perception of intensity of light in, 472, 475; of direction of light in, 471; localization of perception in, 468, 470, 471; in plagiotropic organs, 464, 467; positive, 460; latent period in, 473; primary influence of light in, 474; profile position in, 466; movement in, influenced by stimuli, 462; sequence of stimuli in, 475; liminal intensity of stimulus in, 462, 473; torsions in, *see* Torsions; Weber's Law applied to, 473; wave lengths of light in, 474.
Heredity, 376, 383; material basis of, 376, 377.
Heterotrophic plants, nutrition of, in relation to carbon, 178; to nitrogen, 181; to humus, 183; enzyme formation in, 183; metabolism in, 188.
Hibernation, 341, 348; respiration during, 341.
Holdfasts, 315.
Hook climbers, 455.
Humidity of air, influence of, on transpiration, 39; on growth, 318, 342.
Humus plants, 183, 241.
Humus soils, 100.
Hybrids, hetero- and homo-dynamic, 373; intermediate forms in, 373; new characters in, 374; reversion in, 374; segregation in, 375; sterility of, 376; vegetative power of, 374.
Hydathodes, 57.
Hydrocarbon, as a product of assimilation, 110.
Hydrogen, as a product of fermentation, 218.
Hydrolysis, 150, 152.
Hydrophytes, 253.
Hydrotaxis, 548.
Hydrotropism, 484.
Hygrophilous plants, 390.
Hygroscopic movements, 406.
Hypertrophy, 321.
Hyponasty, 530.

Ice, formation of, 300.
Idioplasm, 377; distribution of, in the plant, 379; in the cell, 377.
Imbibition water, 415.
Impacts, external, in slinging movements, 426, *see* Stimuli.
Impatiens, ejaculatory movements in, 424.
Increment, in growth, 288; maximal, 293.
Indefinite shoot, 277.
Individual variation, 387.
Induced movement, 428, 521.
Influences, external, on growth, 253, 298; correlative, 251, 326; of other organisms, 320.

Inhibiting agents, 156.
Initials, 371; latent, 374, 377, 395.
Inorganic constituents, *see* Ash.
Insectivorous plants, 184; movements in response to stimuli in, see *Drosera*; digestion of proteid by, 185.
Intercalary growing points, 273, 291; growth, 259-63.
Intercellular protoplasmic threads, significance of, for conduction of stimuli, 475; significance of, for translocation of materials, 170.
Intercellular spaces, 37, 104, 120, 199, 296.
Intermittent stimulation, 439.
Intra-molecular respiration, 203; relation to fermentation, 207; products of, 203.
Intussusception, *see* Cell-wall.
Inulin, 162.
Invertase, 152.
Ions, 16.
Iron, 85; -bacteria, 224.

Kinoplasm, 369.
Klinostat, 430; theory of, 438, 449.

Lactic acid fermentation, 217.
Lactose, 210.
Latent characters, 376.
Latent period, 437.
Lateral roots, geotropism in, 446.
Laticiferous tubes, 172.
Leaf-fall, 351.
Leaves, 277; acropetal expansion of, 292; assimilation of carbon-dioxide by the, 107, 251; absorption of ammonia by the, 137; materials of the ash by the, 78; of carbon-dioxide by the, 120; of organic substances by the, 112, 186; of water by the, 33; translocation of materials from the, 162; movements of, due to stimuli, *see* Stimuli; autonomous, *see* Autonomous; etiolation of, 305; light position of, 464, 466; regeneration of, 329; growth of, 292-4; formation of, 281; climbers, 495; arrangement, 275; mechanical theory of, 337.
Leaves, unfolding of, 292.
Lecithin, 4.
Leguminosae, nitrogen fixation in, 237.
Leucin, 140, 144, 174.
Levulose, as a chemical stimulus, 317; *see* Carbohydrates.
Lichen-symbiosis, chemistry of, 243; morphogenic results of, 325.
Life, interpretation of, 254; causes of, 254; conditions of, 298; duration of, 351.
Light, absorption of, in carbon assimilation, 130; influence of, in flower formation, 364; in protoplasmic movement, 540; intensity of, significance of, in carbon-dioxide assimilation, 125; on formation, 306, 310; on growth, 301, 302; production of, 401; quality of, influence of, in carbon-dioxide assimilation, 126; in formation of chlorophyll, 311; in formation, 311; in growth, 310; direction of, influence of on movements, *see* Heliotropism, Phototaxis; influence of, on polarity and symmetry, 310; as inhibiting growth, 303; energy of, in relation to carbon-dioxide assimilation, 130; amount of, made use of, 307; position of leaves, 466; alteration in, influence of, on growth, 343; as a stimulus, *see* Nyctitropism, Phototaxis, Apobatic.
Lime, 98.
Limiting angle, 449; in geotropism, 446.
Lipase, 152, 159.
Lithium, 83.
Locomotion, 405; autonomous, 532; induced, 532, 541.
Longitudinal growth, 286; measurement of, 286; secondary, 295; distribution of, in the stem, leaf and root, 290, 291.

Magnesium, 84.
Malformations, due to mutation, 393.
Malic acid, chemotaxis by, 543.
Maltose, derived from starch, 150.
Manganese, 87.
Mannite, 112, 175, 179.
Manuring, 101.
Mass, movement of water in, 47; of nutrients, 170.
Material and form, 256.
Materials, absorption of, by land plants, 24; by the cell, 11, 24; translocation of, 166; organs concerned in, 169, 170; causes of, 167-9.
Maximum, *see* Cardinal points.
Mechanical stimuli, *see* Pressure, Contact, Shock, Tension.
Mechanism, structure of, 522.
Mechanism and organism, 255.
Merogeny, 371.
Metabolism, 2.
Metamorphosis, 1, 247, 249, 256.
Metamorphosis, due to gall insects, 320; natural, 349.
Metamorphosis of organs, due to correlative influences, 330; due to intensity of light, 310; due to Fungi, &c., 321.
Metaphase, in nuclear division, 268.
Methane, in fermentation of cellulose, 218; derivatives of, as nutrients, 180.
Methods of Physiology, 2.
Micella, 407.
Mimosa, chemical stimulation of, 516; nyctitropic movements in, 504; periodic movements in, 510; movements in response to shock in, 516; significance of, 513; mechanics of, 514; resistance to flexion in, 514; transmission of stimulus in, 518; in response to wounds in, 516.
Minimum, *see* Cardinal points; law of the, 83.

Mixing of initials, 371.
Molecular weight, determined by plasmolysis, 16.
Mosaic hybrids, 373.
Mosses, desiccation of, 317.
Mould-fungi, nutrition of, 178.
Movement, 1, 403; active, 405; autonomous, 428; by torsion, 406; by curvature, 406; by twining, 406; rectilinear, 405; hygroscopic, 410; induced, 428; rotatory, 455; locomotory, 428, 532; paratonic, 428; passive, 405; causes of, *see* Cilia, Cohesion, Protoplasm, Swelling, Turgor, Growth, Shrivelling.
Mucilage, as a reserve, 162.
Multicellular formation, 271.
Mutation, 393.
Mycoderma, 180, 217.
Mycorhiza, 240.
Myxomycetes, alteration of form in, 247.

Narcotics, chemotactical influence of, 542; influence of, on assimilation, 195; on respiration, 195, 202; on *Mimosa*, 516; on perception, 527; on tendrils, 492; on reaction, 527; on development of shoots, 345; want of, as a formal condition, 539; osmosis of, 19.
Nasties, 428, 449, 500, 530; transitions to tropism, 499.
Natatory movements, 532.
Nectaries, 59.
Night position, 500.
Nitrate formation, 228.
Nitric acid, assimilation of, 133; formation of, 226; occurrence of, 135, 136.
Nitrification, *see* Nitrogen-Bacteria.
Nitrogen, assimilation of atmospheric, 232; by autotrophic plants, 133, *see* Ammonia and Nitric acid; in proteid formation, 138; by heterotrophic plants, 181; influence of light on, 142; organic compounds of, 143; atmospheric, 134, 136, 232, 236; combination of, in soil, 233; by *Clostridium*, 233; by Leguminosae, 237; by other Phanerogams, 239; by Fungi, 235; manuring with, 138; gain in, 135; etiolation in absence of, 315; as a nutrient, 86; loss of, 136.
Nitrogen-Bacteria, 228; respiration of, 229; nutrition of, by carbon-dioxide, 228; morphology of, 227; as nitrate formers, 226; as nitrite formers, 226; oxygen requirements of, 226; injury to, by organic substances, 224; occurrence of, 230.
Non-cellular, 258.
Nuclein, in sexual cells, 367.
Nucleus, 6; division of, 268, 271; as the bearer of hereditary characters, 377.
Nutation, 293, 421; simple, 531; transitory, 530; epinastic, 531; hyponastic, 531; periodic, 530; revolving, 530; undulatory, 531.
Nutrients, incombustible, 86; combustible, 86; absence of, influence on growth, 315.
Nutritive solutions for autotrophic plants, 81; for heterotrophic plants, 178.
Nyctitropism, 500; cooling as a stimulus, 502; significance of, 511; in flowers, 501; heating as a stimulus in, 501, 505; of foliage-leaves, 503; alteration in light as a stimulus in, 502, 504; periodic movements in, 509; backward curvature in, 503; influence of gravity in, 508; variation movements in, 504; resistance to flexion in the pulvinus in, 506; mechanics of, 505.

Oenothera, mutation in, 393.
Oil emulsion, 538.
Oils, ethereal, 4, 176; fatty, *see* Fats.
Omnivora, 180, 182.
Opening of flowers, 500.
Optimum, *see* Cardinal points; in inorganic processes, 526.
Orchidaceae, autonomous movements in flowers of, 528.
Organic acids, 4; formation of, by Fungi, 197; by succulents, 198; as nutrients, 178; osmotic activity of, 404; materials, as nutrients in autotrophic plants, 112, 143; in heterotrophic plants, 177.
Organic characters, 386.
Organism and mechanism, 255.
Organization, 9.
Organs, formation of, in regeneration, 331; at growing points, 274, 280, 281.
Orientation, movements, 429.
Orthotropic, 445.
Osmosis, 11.
Osmotaxis, 548.
Oxalic acid, 85, 197.
Oxydases, 204.
Oxygen, loosely combined, 215, 216; need for by anaerobes, 215; for respiration, 192; for movement, 539; for fermentation, 212; for growth, 316; occurrence of in the cell, 196; rigor, 526.

Pangenesis, 391.
Parasites, 178, 186.
Parastichies, 275.
Paratonic movements, 521.
Parthenogenesis, 369.
Partition walls, arrangement of, 270.
Passive layers, 409.
Pectin, 157; fermentation of, 218.
Pepsin, 160.
Peptone, 139, 178; organisms, 182, 241.
Perception, 440, 469; *see* individual stimulating agents; distinct from reaction, 441, 469, 527; apparatus, 441, 443, 523.
Pericline, 280.
Period, grand, 288, 529.

Periodic movements, 508; origin of, 509; mechanics of, 510.
Periodicity, 340; in leaf-formation, 343, 348; in secondary growth, 350; in development, as a whole, 351; in longitudinal growth, 344; yearly, 343–8; daily, 342; in tropical plants, 347.
Performance of work, 247, 397; in geotropic curvature, 437; in imbibition, 408; in turgor and growth, 422.
Permanent tissue, 296.
Petiole, 292.
Phosphorus, 83, 145.
Photonastic, 500, 502, 531.
Phototaxis, 549, 552.
Phototropism, 460.
Physiology, problems of, 1.
Pilobolus, ejaculatory movements of, 423.
Pith, geotropic behaviour of, 435.
Pits, 46, 170.
Plagiotropic, 445.
Plant materials, 4.
Plasmatic layer, osmotic characters, 22.
Plasmodia, movements of, 534; chemical composition of, 8.
Plasmolysis, 14.
Plastic stretching, 265.
Pneumathodes, 38.
Poisons, effect of, on growth, 316; as stimuli, 87.
Polarity, 330, 333, 336.
Poles, 273.
Pollen-tube, conditions of germination of, 317; directive movements in, 483.
Polygonum amphibium, adaptation in, 253.
Posterior side, 276.
Potassium, 83.
Potometer, 30.
Precipitation membranes, 13.
Predominant characters, 373.
Pressure, influence of, on organogenesis, 338; on lateral roots, 315; on division planes in the cell, 314; on growth and formation, 314; negative, of the air in vessels, 70; osmotic, in external medium, 179, 418; variations in, 318; significance of, 20, 419; determination of, 18, 420; amount of, 18, 419; regulation of, 419; theory of, 17; effect of, 420; as a stimulus, 314, *see* Contact; exerted by the growing plant, 422.
Primitive organisms, 258.
Production of energy in the plant, 397–404.
Progressive development, 274.
Prophase, in nuclear division, 268.
Protease, 152, 160.
Proteids, 4, 140; formation of, 115, 138; from amido-compounds, 173; chemistry of, 139; subdivision of, 140; as reserves, 159, 163, 165; decomposition of, 139, 161; respiration of, 200; fermentation of, 219; organisms, 182.
Protein, *see* Proteid.
Protoplasm, 6; amoeboid movements of, 534; explanation of, 537; formal conditions of, 539; rotation of, 536; circulation of, 536; permeability of, 19, 20; causes of, 21; structure of, 9, 10; growth of, 259; composition of, 7.
Putrefaction, 219.

Qualitative division, 380.
Qualities, mechanical explanation of, 254.

Racemic acid, 181.
Races, 384, 388.
Radial growing point, 276.
Ranunculus aquatilis, adaptation in, 253.
Reaction, *see* Perception; purposeful, 254; forms of, 428.
Recessive characters, 373.
Reduction division, 367, 378.
Reflex actions, 525.
Regeneration, 328–9; from the single cell, 380; from somatic cells, 380; causes of, 336.
Rejuvenescence, 366, 371.
Releasing agents, 255, 339, 428, 522, 527.
Reproduction, 353; in Algae, 353; in *Basidiobolus*, 249; digenous, 371; in ferns, 358; sexual, 249, 355; by embryos and buds, 355; monogenous, 371; in Phanerogams, 360; asexual, 249, 355; causes of, 355; relationship of, to growth, 357; organs of, 353; accessory, 359, 362.
Reproductive-leaves, 349.
Reserve organs, 329.
Reserves, 147, 148, 161, 162.
Resins, 4.
Resistance to flexion in articulations, 506, 514.
Respiration, 190; dependence of, on developmental condition, 194; on light, 201; on oxygen, 202; on material influences, 201; on temperature, 201; on water, 341; significance of, 205; history of our knowledge of, 205; amount of, 193; intramolecular, 203, 316; coefficient of, 197, 199; dependent on materials, 199; and production of light, 401; materials used in, 196, 208; demonstration of, 192; in the green cell, 194; primary decomposition in, 204; products of: alcohol, 204; carbon-dioxide, 192; organic acids, 197; water, 196; causes of, 203, 204; occurrence of, 191; production of heat in, 201, 400.
Rest period, 345.
Retting, 218.
Reversible enzyme actions, 154.
Rheotaxis, 548.
Rheotropism, 486.
Rhinanthaceae, parasitism in, 188.
Rhizomes, geotropism in, 446, 448.
Rigidity, of the stem, 251; of the cell, 251, 419.

Rigor, 526.
Ringing, 48, 171.
Root, conditions of origin of, 338; functions of, 26; size of, 28; periodicity of growth in, 346; branching of, 26, 97, 283; absorption of water by, 28; growth of, 289; corrosion figures of, on limestone, 94; pressure, *see* Bleeding; hairs, 28; functions of, 93, cap, 283; tendrils, 495.
Rotation, 536.
Rubidium, 83.

Saccharomyces, as causing alcoholic fermentation, 208.
Saprophytes, 178.
Scale and foliage-leaves, 349.
Scion, 334.
Secondary growth, 294; eccentric, 314; contraction during, 295.
Secretion of peptic enzymes, 184; of acids, 184; of water, 50, 57.
Seeds, *see* Germination.
Segment cells, 280.
Segregation in hybrids, 375; in *Cytisus adami*, 381.
Selection, 384–6; of nutrients, 180.
Self-regulation, phenomena of, 184, 255, 525.
Semipermeability, 12, 13.
Sexual-cells, injurious influence of, 376.
Shock-stimuli, 516; in Cynareae, 519; in *Mimosa*, 511; in styles, 520; in other structures, 517, 520.
Shoots, sprouting of, 290, 344, 345; elongation of, 290; development of, 345, 346.
Shrivelling, movements due to, 409.
Sieve-tubes, 172.
Silica, 87.
Siliciferous plants, 99.
Silicon, 87.
Size, specific, 340.
Soap films, 270.
Sodium, 83, 86.
Soil, absorption by, 92; employment of, by the root, 26, 96; occupation of, by plants, 97: origin of, 90; nutrients in the, 91; oxygen in the, 25; primitive, 90; alluvial, 90; water contents of, 25; and agriculture, 101; and plant distribution, 97; plants local and indifferent to, 97.
Solution, theory of, 16; colloidal, 153.
Soma, somatic, 273.
Somatic cells, initials of, 380.
Specialists, 180, 182.
Species, definition of, 384; primary or sub-, 385; origin of, by mutation, 393; origin of, according to Darwin, 384; characters of, 385.
Spermatozoa, 359, 543.
Spindle threads, 268.
Spiral arrangement of members, 275.
Spiral, fundamental, 275.
Spontaneous movements, 521.
Sporangium, dehiscence of, 415.
Spores, ejection of, 422.
Spring plants, periodicity in, 351; shoots of, 346.
Stamens, movements of, 425, 520.
Starch, as a product of assimilation, 110; translocation of, 147; formation of, from sugar, &c., 112; transitory, 171; dissolution of, 149.
Statocysts, statoliths, 442.
Stem climbers, 495.
Stigma, stimulation of, by shock, 520.
Stimuli, 255, 298, 496; in general, 427, 522; chemical, &c., *see* the individual stimuli; special, 427; specific, 522; application of, 524; movements in response to, general characters of, 521; chain of, 525; intensity of, in relation to reactions, 526; transmission of, in *Drosera*, 498; in heliotropism, 468, 469, 470, 475; in *Mimosa*, 518; in tendrils, 493; media of, 428, 524; materials acting as, 88; reaction to, 428.
Stock, 334.
Stomata, 37; absorption of carbon-dioxide by, 120; structure of, 38; mechanics of, 39; opening of, effect of external factors on, 40–42.
Stretching of the cell-wall, due to osmotic pressure, 419.
Strontium, 85.
Structure, specific, 339.
Style, sensitive, 520.
Stylidium, autonomous movements in, 529.
Suberin, 4.
Substance, embryonic, 273.
Substratum, orientation in reference to, 486.
Succinic acid, in fermentation, 210.
Sugar, formation of, from fat, 199; promoted by low temperatures, 175.
Sulphur, 83, 145; in *Beggiatoa*, 220; Bacteria, colourless, 223; new group of, 229; red, 223.
Sulphuretted hydrogen, 221.
Sulphuric acid, assimilation of, 145; formation of, by *Beggiatoa*, 221.
Superelongation in darkness, 305; in absence of nitrogen, 315.
Surface tension, 537.
Surfaces of minimum area, 270.
Swarmspores, 354.
Swelling, 406.
Symbiosis, 237, 325.
Symmetry, 274.

Taxis, 541.
Temperature, influence of, on growth, 299; as a formal condition, 252, 526, 540; formative effect of, 301; cardinal points of, 300, 526; of the plant, 399; alteration in, effects of, on growth, 342; as a stimulus to movement, 500, 505; reduction

of, by conduction, 398; by radiation, 398; by transpiration, 44, 398.
Tendrils, reacting all round, 490; senile curvature in, 495; autotropism in, 493; reacting unilaterally, 489; distribution of sensitivity in, 490; curvature in, on stimulation, 499; circumnutation in, 489, 530; perception in, 490, 491; stimulation of both sides of, 490; due to chemical influences, 492; due to electricity, 492; due to contact, 491; due to temperature, 492; tropistic curvature in, 490; clasping of supports by, 495; distribution of growth in, 488; nastic curvature in, 499.
Tension, influence of on growth, 261.
Tetrathionic acid, 229.
Thermonastic, 499, 500, 502.
Thermotaxis, 548.
Thigmotaxis, 548.
Thiosulphate, 229.
Thiothrix, 223.
Thyloses, 296.
Tissue tensions, 297, 421.
Tonotaxis, *see* Osmotaxis.
Torsion, 406; autonomous, 531; geotropic, 452, 453; heliotropic, 465; due to shrivelling, 414.
Tracheae and tracheides, *see* Vessels.
Transitory nutation, 530.
Translocation products, 148, 172; of materials: from the leaf, 166; from storehouses of reserves, 167; from seeds, 155, 168.
Transpiration, 25, 35; dependence of, on external conditions, 38; on stomata, 39; on the structure of the plant, 37; influence of, on absorption of nutrients, 43; on the temperature of the plant, 43; on ascent of water, 74; acceleration of, 43; formative results of, 317; amount of, 43; significance of, 43; inhibition of, 42; internal, 37; cuticular, 38; stomatal, 38.
Transplantation, 332.
Traumatotaxis, 553.
Traumatotropism, 486.
Trees, annual periodicity in, 344; winter transformation of starch into fat in, 175.
Tropism, 428, 499.
Turgor, 13, 263, 418.
Twining plants, 455; geotropism in, 457; spiral movements of, 456; supports to, 458; torsions in, 458; twining of, 455.
Twinings, 406; autonomous, 530; in tendrils, *see* Tendrils; due to shrivelling, 412; in twining plants, 457.
Tyrosin, 140, 174, 444.
Undulatory nutation, 531.
Unicellular, 272.
Urea, as a nutrient, 143, 144; fermentation of, 224.
Uro-Bacteria, 224.
Urticaceae, stimulus movements in, 425.

Vacuoles, 6.
Variation, *see* Adaptation, Species, Mutation; fluctuating, 387; movements, 421; autonomous, 528; geotropic, 453; heliotropic, 465; paratonic, 428.
Vegetative growing point, 272; basal, 273; structure of, 279–81; form of, 276; intercalary, 273, 292; of the stem, 277; symmetry of, 274, 276; capacity of, 273; terminal, 273; growth in, 280; distribution of, 284; of the root, 283; arrangement of cells in, 279.
Ventral, side, 276.
Vessels, structure of, 66–70; functions of, 47, 172; contents of, 70; absorption of water by, 49.
Volume, alteration in, in growth, 258.

Water, excretion of, *see* Bleeding, Transpiration; absorption of, by aerial organs, 32; by roots, 29; by cells, 24; influence of different factors on, 31; excretion of, by hydathodes, 58; significance of, in movement, 539; movement of, in the plant, 45–7; influence of, in germination, 252; in growth, 317; withdrawal of, 318; conduction of, 25; influence of living cells on, 74–6; through vessels, 47; by capillarity, 72; rate of, 62; height of, 62; amount of, 61; by parenchyma, 46; direction of, 61; by suction, 64; in dead trees, 75; by root pressure, 63; glands, 58; amount of in soil, 26, 318; in air, 318; capacity, 26; culture, 80; plants, absorption by, 24; cause of form, 319.
Wax, 4.
Whorl, 274.
Working energy, 205, 403, 404.
Working materials, 148.
Wounds, healing of, 328; regeneration from, 329; as stimuli to protoplasmic movement, 540; as directive stimuli, 486, 553.

Xerophytes, adaptation in, 253.

Zinc, 87.
Zone of maximal growth, 289.
Zymase, 211.

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